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(54) Title: BIOCOMPATIBLE HYDROXYAPATITE FORMULATIONS AND USES THEREFOR

(57) Abstract

A biocompatible hydroxyapatite formulation is provided. The formulation is precipitated from a mixture of a liquid phase, a bioactive or biocompatible additive which may be any of a number of bioreactive or other substances, and a base combination of calcium phosphate salts. The liquid phase and the additive may be combined to produce an augmented liquid phase, which is then mixed with the base salt combination. The additive is chosen to achieve a desired effect during administration of the formulation to a plant or animal. The additive is released into the surrounding physiological milieu and the hydroxyapatite component is resorbed.

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BIOCOMPATIBLE HYDROXYAPATITE FORMULATIONS AND USES THEREFOR

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention generally relates to certain combinations of sparingly soluble calcium phosphates and applications thereof. More specifically, certain calcium phosphates may be combined with a liquid to form a paste or slurry of hydroxyapatite, which has a variety of medical, dental and other uses. Various biocompatible additives may be incorporated into the paste or slurry for various applications.

2. Description of the Related Art

Hydroxyapatite is a calcium phosphate mineral and is the primary constituent of human bones and teeth. Hydroxyapatite is only one of a number of such calcium phosphate minerals, which have differing calcium-to-phosphate ratios, crystal structures, and physical characteristics. Apatite is a general term for a wide range of compounds represented by the general formula $M^{2+}_{10}(ZO_4^{3-})_6Y^-_2$, where M is a metal atom, particularly alkali or an alkaline earth metal atom, and ZO_4 is an acid radical, where Z may be phosphorous, arsenic, vanadium, sulfur or silicon, or may be substituted in whole or part with carbonate (CO_3^{2-}). Y is an anion, usually halide, hydroxy or carbonate.

A combination of sparingly soluble calcium phosphate salts, especially tetracalcium phosphate and another sparingly soluble calcium phosphate salt, both in their solid state, in equilibrium or quasi equilibrium in an aqueous or non-aqueous liquid phase such that both salts are in excess, can precipitate hydroxyapatite, i.e., $Ca_5(PO_4)_3OH$. If both calcium phosphate salts are near equilibrium with the same saturated solution which additionally is supersaturated with respect to hydroxyapatite, then the composition will continue to precipitate hydroxyapatite. The precipitated hydroxyapatite can be formed either in vivo or ex vivo (in vitro) and may possess varying mechanical and biological features including, e.g., hardness, flexibility, porosity, dissolution,

bioresorption, biodegradation, tissue adhesion, and replacement by soft and hard tissues.

Hydroxyapatite, as well as modified forms thereof, is a naturally occurring material in bone, teeth and some invertebrate skeletons. Crystals of hydroxyapatite are embedded in the matrices of bone and tooth together with cells and tissue matrix materials including fibrous proteins such as cross-linked collagen and mineral binding proteins such as certain *gla* proteins, dentin and enamel. All vertebrate and dentulous animals are capable of causing mineralization of bone and tooth matrices through the precipitation of hydroxyapatite crystals under suitable physiological conditions of pH, temperature and ionic conditions. The resulting tissues are not highly cellular and display certain unique properties, such as significant mechanical strength, flexibility, physiological activity, and continual self-remodeling.

Because of its unique properties, hydroxyapatite is a highly biocompatible material. These unique properties of hydroxyapatite present in bone have prompted efforts to develop implants made of hydroxyapatite, ceramics and other similar hard calcium phosphate materials such as α- and β-tricalcium phosphates. These implants have been used to fill a wide range of bony defects in orthopedic and reconstructive surgeries and in the anchoring of tooth to bone (e.g., in periodontal, dental and orthognathic applications). Many physical and chemical variations have been attempted to create implants (i) with increased mechanical strength in order to enable use of hydroxyapatite alone or hydroxyapatite composite implants in load bearing bone defect sites; (ii) with altered porosity to allow better bone ingrowth such that the implant is effectively incorporated in the newly formed bone tissue; and (iii) as granular forms to allow packing in surgical defect sites. In addition to these applications, biological factors that are believed to cause the formation and growth of various

cells implicated in bone formation have been used to produce hydroxyapatite composite implants with "inductive" properties.

Hydroxyapatite has other known applications, such as bone repair and remineralization of teeth. Such uses are shown in, for example, U.S. Patent Nos. Re. 33,221 and Re. 33,161 to Brown et al. One specific example is the repair of tooth lesions or cavities. When an incipient lesion or cavity develops on the surface of a tooth, the dentist traditionally fills the cavity that forms. This procedure may prevent the decay from spreading further, but does not restore the tooth to its original state. A considerable amount of research, however, has been directed to the mineralization of incipient dental lesions. The object of remineralization is to deposit hydroxyapatite on the carie lesion such that the dental enamel incorporates the hydroxyapatite into its structure at the point of lesion. Thus, remineralization prevents further tooth decay and restores the tooth. Generally, the supersaturated solutions or slurries used for remineralization have been prepared from a single form of calcium phosphate. However, these solutions or slurries have been unsatisfactory for a variety of reasons. For example, the amount of calcium and phosphate ions available for remineralization in these supersaturated solutions is relatively low, thereby requiring both an excessive volume of fluid and an excessive number of applications.

The use of a single calcium phosphate, β—$Ca_3(PO_4)_2$, was suggested for pulp capping in Driskell et al., "Development of Ceramic and Ceramic Composite Devices for Maxillofacial Application," J. Biomed. Mat. Res. 6: 345-361 (1972) and the use of $Ca_4(PO_4)_2O$ was suggested by the inventors in IADR Abstract. No. 120, J. Dent. Res. 54: 74 (1975) as a possible pulp capping agent. These single calcium phosphate cements, however, are incapable of setting to a hard consistency and suffer the same drawbacks as

single calcium phosphate remineralizers. For example, they cannot maintain a relatively constant pH and do not have sufficient remineralization capacity.

Experience with calcium-based implants for the replacement of skeletal tissue has also existed for many years. Most of these implants have been in the form of prefabricated, sintered hydroxyapatite in either granule or block form. These preparations have several drawbacks, including a limited ability to conform to skeletal defects, particularly in the case of blocks; inadequate structural integrity of granules (which do not bond together); and difficulty in modeling into an implant having the shape of missing skeletal tissue.

Various forms of hydroxyapatite, methods of their production, coating of metallic or other prosthetic devices with hydroxyapatite, coformulation with other polymeric substances, admixing with biological tissue structural components such as collagen, combination with certain pharmaceutical agents of diagnostic or therapeutic uses, and mixing with biologically active proteins or polypeptides have been described in the scientific and patent literature. Since all such disclosures would be too numerous to list, certain examples relevant to the present invention are cited herein. U.S. Patent Nos. 4,795,467; 4,865,602; 4,992,226; 5,123,925; and 5,246,457 disclose mineral-collagen mixture preparations, including methods of sterilization, for bone repair wherein the mineral component can be selected from a group of tricalcium phosphates or hydroxyapatites with preferred particle size of between 100-2000 μm and the collagen is either mixed to provide a moldable formulation or applied to coat the porous interstices within the ceramic particles. U.S. Patent No. 5,204,382 and International Application WO 93/16657 disclose injectable compositions comprising particulate ceramic materials with sizes between 50-250 μm and collagen or another biocompatible organic polymer. These combinations are intended for repair and augmentation of hard or soft tissues through the dissipation of the biocompatible component leaving behind the

ceramic particles. The patent literature also describes admixing of biological factors implicated in the regeneration of bone with hydroxyapatites (including coralline hydroxyapatite originally described by Holmes et al. in 1979 - Plastic and Reconstructive Surgery volume 63, page 626) or tricalcium phosphate granules and the uses of such formulations in bone repair. All of these disclosures relate to materials wherein the hydroxyapatite is preformed (as octacalcium phosphate or β-tricalcium phosphate salts) and is substantially composed of or manufactured from one essentially homogeneous form of calcium phosphate salt with contaminants or impurities present due to or arising from the raw material source or the manufacturing method.

In Re. 33,221 and Re. 33,161, Brown and Chow have described powders, pastes and slurries comprising sparingly soluble calcium phosphate salts, one of which is anhydrous tetracalcium phosphate, that are capable of precipitating hydroxyapatite under ambient temperature conditions in a wide range of liquid phases. In U.S. Patent Nos. 4,880,610; 5,047,031; 5,053,212; 5,129,905; and 5,178,845 and in International Applications WO 92/02543, EP 0,347,028 A2, and EP 0,416,761 A1, Constantz et al. have disclosed certain in situ calcium phosphate mineral methods, intimate mixtures of calcium and phosphate, wherein the source of phosphate is phosphoric acid in a substantially anhydrous form, compositions obtained thereby, and their use in bone repair. Constantz et al. also have described uses of such materials in coating of bone prostheses. See, U.S. Patent Nos. 5,164,187 and 5,188,670, and International Application EP 0,383,568 A2. U.S. Patent Nos. 5,034,059 and 5,231,169, and International Application WO 93/12736 disclose combining and/or mineralizing collagen to produce physical characteristics resembling bone. JP 1,111,762 discloses a powder or a powder mixture containing tetracalcium phosphate and a kneading solution to produce a hardening composition which produces hydroxyapatite on contact with water. EP 436,499 provides a process for

producing a calcium phosphate-type powder, as a highly active powder, as a component in calcium phosphate-type hardening materials. These materials may be used to obtain a highly plastic, moldable material that hardens under mild conditions. In U.S. Patent No. 5,068,122, Kokubo et al. have described a process of forming a bone-like bioactive film of hydroxyapatite on a desired substrate surface without the use of heat treatment and through the precipitation of hydroxyapatite from solutions containing a certain range of Ca^{2+} and HPO_4^{2-} ions. Liu et al. have described the use of tetracalcium phosphate alone, or together with α-tricalcium phosphate as a base, to produce calcium phosphate cements with relatively high surface pH that are considered beneficial for their biocompatibility in orthopedic, dental and maxillofacial applications. See, U.S. Patent No. 5,149,368. Unlike the preformed hydroxyapatites, calcium phosphate cements comprising the various precursor calcium phosphate salts and capable of precipitating hydroxyapatite provide the option of incorporation with bioactive substances such as proteins to produce implants that induce active regeneration of tissues such as bone. Incorporation with tissue matrix proteins, such as collagen, may result in implants that are desirable matrices for growth of tissues, including bone and soft tissues. However, these references only concern incorporation of bioactive or biocompatible proteins and related substances as a means to improve the properties of hydroxyapatite as an implant material for tissue repair applications.

Numerous patents, patent applications and publications describe instruments and their uses for and methods of influencing tissue behavior, including healing by the use of electrical and electromagnetic fields. Invasive implants that serve as electrodes as well as externally placed electrodes have been used to generate electrical or electromagnetic fields of direct or pulsating nature in and around selected tissue sites. The reported results include augmentation or healing of bone fractures, relaxation of muscle fibers,

regeneration of soft tissues, and the like. One of the earliest related patents is U.S. Patent No. 3,055,372. Several specific designs of devices with superior utility in specific applications have since been described including, for example, devices used in the promotion of ingrowth of bones into pores of hydroxyapatite implants. See, e.g., Shimizu et al., Journal of Orthopedic Research vol. 6, pgs. 248-258. However, none of these references discloses a formulation or device designed to modulate and/or enhance mechanical effects or the effects of electrical stimulation in causing healing.

The available technologies and products, which utilize hydroxyapatite suffer from other conceptual as well as practical limitations. One of the major conceptual limitations arises from the focus on using hydroxyapatite for tissue replacement as described above. Typically, the implant is intended to either fill a tissue void and provide structural support or to be incorporated into newly formed tissue developed during the process of repair or regeneration. As a consequence, little effort has been devoted to utilizing the biocompatible properties of hydroxyapatite and developing formulations whereby the hydroxyapatite is resorbed or degraded in a manner similar to certain synthetic polymers and principally functions as a biocompatible matrix to localize physiologically active materials at a predetermined site, or to achieve the presentation of such materials in a physiological milieu in a controlled manner resulting in enhanced utility. The practical limitations in pursuing the novel formulations of the present invention arise from several significant technological limitations.

First, current hydroxyapatite technology is limited by harsh production methodology. Most hydroxyapatite implants are produced under extreme physical and chemical conditions resulting in hard implants comprised of large, fused, macro-crystals with random pores and gaps, unlike material formed naturally in bone or other skeletal structures encountered in nature.

Second, typical hydroxyapatite formulations are characterized by limited tissue compatibility and resorbability. For instance, the hardness and the nature of the crystal structure frequently make them incompatible for application in repairing soft tissues. These shortcomings are caused not only by the physical characteristics of the formulations, but are also due to the body's inability to completely resorb the material over an acceptable time period. Third, known formulations also suffer from limited coformulation capability. Biologically active factors that can promote cell growth or biocompatible substances that offer a better support for cell growth can only be applied to the surface of conventional hydroxyapatite implant by spraying, freeze drying or soaking since the conditions used to produce the implants generally destroy biological activities. Fourth, known formulations may have undesirable release characteristics. Biocompatible or bioactive materials applied to coat a hydroxyapatite implant surface may be rapidly washed away in a physiological milieu resulting in a rapid rise in local concentration of the substance with little or no material remaining available for the long term action required for most of these substances. Other shortcomings of current hydroxyapatite technology exist.

SUMMARY OF THE INVENTION

The biocompatibility of hydroxyapatite and the ability to precipitate hydroxyapatite crystals under ambient conditions leads to the production of novel biomaterials wherein biologically active substances may be incorporated. These biomaterials are also amenable to coformulation with or coating of other polymeric (e.g., dacron, nylon, vinyl, teflon, acrylic), elemental (e.g., carbon, titanium), or alloy (e.g., steel) implants. Novel biomaterials selected based on mechanical and biological properties have utility in providing structural support for tissue filling or covering, or the stabilization of other mechanical devices; in promoting or supporting tissue formation for repair,

regeneration or augmentation of tissues; in delivering or releasing antigenic materials for vaccination or genetic materials for gene therapy; in facilitating local, extended or controlled delivery of pharmaceutically active agents such as antibiotics or chemotherapeutics or hormonal substances for enhanced therapeutic effect; and in providing a piezoelectric field to deliver or amplify direct or pulsed electromagnetic stimulation to enhance healing or to generate controlled mechanical motion. These are merely examples of applications of a biocompatible hydroxyapatite formulation.

Based on the consistency and the desired site of application, the formulations and methods of this invention will be amenable to different means of administration (e.g., injection, implantation, arthroscopic application, packing, etc.) in different medical applications. As discussed above, the molecular composition of hydroxyapatite makes it highly biocompatible. A significant portion of an adult vertebrate animal's body weight is comprised of calcium phosphate. Since bone is an actively remodeling tissue, there is a substantial turnover of calcium phosphate in a continuous manner. Except for conditions of significant hypercalcemia brought about under certain pathological conditions, the body's major organs are capable of withstanding physiological calcium phosphate turnover, and the excretory system, primarily the kidney, is capable of processing the released calcium phosphate. Harnessing the biocompatibility of calcium phosphate in creating implants (or calcium phosphate compositions that can form implants in situ), which are formed through the precipitation of hydroxyapatite under ambient or physiological conditions and are resorbed at different rates within the body, has a broad range of medical applications.

Formulations based on such biocompatible materials are preferable over synthetic polymers such as polymethylmethacrylate, polyglycolic acid, polylactic acid and copolymers of these substances that are not found in

nature. Although many of these polymers are available in a range of consistencies, the processes of polymerization and depolymerization of these materials are frequently accompanied by unacceptable changes in pH or temperature, may require the use of toxic organic solvents, or lead to undesirable inflammatory reactions in animals and humans.

Hydroxyapatite crystals also possess unique piezoelectric characteristics which can be exploited in modulating the growth and metabolic activities of cells. Therefore, the use of electrical and electromagnetic stimulation approaches for tissue healing or the generation of controlled mechanical motion may be significantly enhanced through the use of implants made of hydroxyapatite crystals. Such implants may also be enhanced by incorporation of other biocompatible or bioactive substances involved in cell manipulation.

It is an object of the present invention, therefore, to provide controlled presentation of a wide variety of biologically active or biocompatible substances at selected locations and gradual delivery of such substances into the local milieu or systemically over desirable time periods that may be appropriate to derive improved clinical benefits compared to other means of administration of such substances.

It is another object of the present invention to provide a formulation of a versatile delivery matrix that is composed of a biocompatible additive, which is amenable to alterations in setting time under physiological and ambient conditions, which is amenable to alterations in the rate of biodegradation or resorption in an animal or human body, which is capable of producing materials with a wide range of consistencies (e.g., slurry, paste, hard granule, block, powder, etc.), and which is able to harden as well as dissolve without significant changes in temperature or pH.

It is a further object of the present invention to provide a formulation of an implant or implantable composition which displays an appropriate combination of resorbability, cell growth supporting capability, and piezoelectric conductance capacity for use in the enhancement of electrical or electromagnetic stimulation.

To achieve these and other objects, the present invention provides formulations of certain sparingly soluble calcium phosphate salt combinations, having the ability to precipitate hydroxyapatite under physiological and/or ambient conditions, wherein a biocompatible or a bioactive material is incorporated during the precipitation process such that the incorporated material is retained at the site of application of the formulation for extended time periods or gradually released into the surrounding physiological milieu. The composition of the salt combination used in a formulation of the present invention is chosen such that the physical properties (porosity, tensile and flexural strength, consistency, etc.) and the resorption/biodegradation properties of the resulting hydroxyapatite precipitate enable the formulation to enhance the function of the incorporated biocompatible or bioactive material.

In an embodiment of the present invention, a biocompatible hydroxyapatite formulation is provided in which hydroxyapatite precipitates under physiological conditions (including pH, temperature, ionic strength, etc.) or under conditions that (i) preserve the activity of an added biocompatible or bioactive substance and (ii) allow the substance to be impregnated reasonably uniformly throughout the precipitated composite. One aspect of this embodiment concerns formulations containing biologically compatible materials that support the growth or formation of different cells or tissues and biologically active substances that cause or induce the formation of such cells or tissues. Another aspect contemplates formulations containing biologically active substances that kill diseased or undesirable cells or tissues, or activate certain cells or tissues

wherein the activity may produce a therapeutic benefit. The biocompatible hydroxyapatite formulations may be used in products and structures with other technologies to create novel combined medical devices.

In another embodiment, a method is provided for preparing a biocompatible hydroxyapatite formulation. A base combination of calcium phosphate salts is prepared. A liquid phase is prepared. A biocompatible additive is provided. These components are then combined to form a mixture. Then, a biocompatible hydroxyapatite formulation is precipitated from this mixture. To form the mixture, any two of the components may be combined first by suitable methods and then combined with the third component. Alternately, the three ingredients may be simultaneously combined. According to various aspects of this embodiment, the biocompatible additive may be any of several additives including, without limitation, growth factors, adhesive agents, immunogens, vaccines, genes, recombinant cells, antibiotics, pharmaceutical agents, hormones, fibers, gels, space occupying particles, and electrical stimulus enhancers. A formulation prepared according to this method may also incorporate a pharmaceutically acceptable carrier.

In another embodiment, a method is provided for treating a patient. In this embodiment, a biocompatible hydroxyapatite formulation is prepared as before. Then, the formulation is administered to the patient.

Further objects, features and advantages of the present invention will readily apparent to those having ordinary skill in the pertinent art from the detailed description of the preferred embodiments with reference to the appropriate figures.

BRIEF DESCRIPTION OF THE DRAWING

Fig. 1 is a plot of the solubility isotherms of $Ca_4(PO_4)_2O$; $CaHPO_4 \cdot 2H_2O$; $CaHPO_4$; $Ca_8H_2(PO_4)_6 \cdot 5H_2O$; β—$Ca_3(PO_4)_2$; and $Ca_5(PO_4)_3OH$ at 25°C in the ternary system of $Ca(OH)_2$; H_3PO_4; and H_2O.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is generally directed to selected mixtures of calcium phosphate salts formed as powders, slurries or pastes and having various mechanical properties, porousness, piezoelectric features and bioresorption characteristics. These materials serve as novel delivery vehicles for various chemical agents used as traditional pharmaceutical drugs and for biological agents including glycoproteins, polymeric hydrocarbons, lipids, glycolipids, carbohydrates, proteins and nucleic acids. Additionally, these materials enhance the effects of electrical or electromagnetic stimulation in tissue healing.

The resulting novel compositions have utility in a broad range of medical applications such as immunization or vaccination, gene therapy, therapeutic modalities mediated through selective elimination or modification of cells or removal of substances from a physiological milieu, repair or regeneration of biological structural material (e.g., bone, muscle, skin, tendon, and ligament) anchoring of tooth to bone, anchoring of prosthetic devices, or augmentation of soft tissues such as breast tissue. Other uses are envisioned and certain specific applications contemplated for the various compositions are discussed in detail below.

The major components for the biocompatible hydroxyapatite formulation comprise sparingly soluble calcium phosphate salts. Preferably, two salts are combined, one of which is preferably tetracalcium phosphate. The other salt may be selected from the group consisting of $CaHPO_4 \cdot 2H_2O$; $CaHPO_4$; $Ca_8H_2(PO_4)_6 \cdot 5H_2O$; β-$Ca_3(PO_4)_2$; α-$Ca_3(PO_4)_2$; and modified $Ca_3(PO_4)_2$, e.g., tri-calcium phosphate modified by protons or up to approximately 10wt% magnesium.

The fundamental principles underlying the selection of the second sparingly soluble calcium phosphate salt that is to be mixed with tetracalcium

phosphate are described by Brown and Chow. See, e.g. U.S. Patent Nos. Re. 33,221 and Re. 33,161; W.E. Brown and L.C. Chow, "J. of Dental Research," vol. 63, p. 672 (1983); W.E. Brown and L.C. Chow, "Cements Research Progress - 1986, American Ceramic Society," p. 352 (1986); and Y. Fukase, E.D. Eanes, S. Takagi, L.C. Chow and W.E. Brown, "J. of Dental Research," vol. 69, p. 1852 (1990). These materials are incorporated by reference.

Basically, each of these calcium phosphate salts has a characteristic solubility behavior that may be represented by a plot of the total concentration of calcium ions at the point of saturation versus the pH of the solution at a constant temperature. A plot of the total concentration of phosphate ions versus pH would be equivalent for the purposes of the present invention because the concentrations of phosphate and calcium ions in solution are linked. The resulting curve is called an isotherm.

When the isotherms for various calcium phosphate salts are plotted on the same axes, their solubility behavior relative to each other may be determined. Specifically, a calcium phosphate whose isotherm lies above the isotherm of another calcium phosphate at a given pH is metastable with respect to the latter. The point where the isotherms of two calcium phosphates intersect is known as a singular point. In a solution that is saturated with respect to the two calcium phosphates, both calcium phosphates will be in equilibrium with the saturated solution at the singular point. This means that neither calcium phosphate will precipitate out of solution, but another calcium phosphate whose isotherm lies below the singular point can precipitate. The present invention in one aspect relates to combinations of calcium phosphate salts that form singular point solutions that are supersaturated with respect to hydroxyapatite.

Fig. 1 is a plot of the solubility isotherms for six calcium phosphate salts in the ternary system comprising $Ca(OH)_2$, H_3PO_4 and H_2O at 25°C. The y-axis of Fig. 1 represents the total concentration of calcium ions in

solution in moles per liter, while the x-axis represents pH. The isotherms for $CaHPO_4 \cdot 2H_2O$, $CaHPO_4$, β—$Ca_3(PO_4)_2$ and $Ca_5(PO_4)_3OH$ are based, respectively, on the following articles: Gregory et al., "Solubility of $CaHPO_4 \cdot 2H_2O$ in the System $Ca(OH)_2$ — H_3PO_4 — H_2O at 5°, 15°, 25° and 37.5°C.," J. Res. Nat. Bur. Stand. 74A: 461-475 (1970); McDowell et al., "Solubility Study of Calcium Hydrogen Phosphate Ion Pari Formation," Inorg. Chem. 10: 1638-1643 (1971); Gregory et al., "Solubility of β—$Ca_3(PO_4)_2$ in the System $Ca(OH)_2$ — H_3PO_4 — H_2O at 5°, 15°, 25° and 37°C.," J. Res Nat. Bur. Stand. 78A; 667-674 (1974); and McDowell et al., "Solubility of $Ca_5(PO_4)_3OH$ in the System $Ca(OH)_2$ — H_3PO_4 — H_2O at 5°, 15°, 25° and 37.5°C.," J. Res. Nat. Bur. Stand. 81A: 273-281 (1977). The isotherm for $Ca_8H_2(PO_4)_6 \cdot 5H_2O$ is based on the solubility product disclosed in Moreno et al., "Stability of Dicalcium Phosphate Dihydrate in Aqueous Solutions and Solubility of Octacalcium Phosphate," Soil Sci. Soc. Am. Proc. 21: 99-102 (1960). The isotherm of $Ca_4(PO_4)_2O$ is based on the approximate value of the solubility product calculated by Brown and Chow as referred to, for example, in Re. 33,221 and Re. 33,161.

In the preferred embodiments of this invention, the ratio of tetracalcium phosphate to the other salt as well as the relative particle sizes are varied in the different contemplated formulations in order to obtain the ones that meet the mechanical criteria and resorption and degradation profiles desired for the particular bioactive or biocompatible substance incorporated as an additive. Additionally, the relative amount of a liquid phase (preferably chosen from the group consisting of water, saline, a weakly acidic solution, a biocompatible buffer solution, serum, plasma, and other bodily fluids) to the amount of the salt combination may be varied in order to alter setting time and the consistency of the resulting precipitated biocompatible hydroxyapatite formulation. The biocompatible or the bioactive additive is preferably dissolved or essentially

uniformly mixed in the liquid phase prior to adding the liquid phase to the dry salts combination and initiating the hydroxyapatite precipitation reaction. Alternatively, the liquid phase may be first combined with the calcium phosphate salts, followed by incorporation of the additive into the mixture. In another alternative, the bioactive additive is first combined with the salts, and then the liquid phase is added. In still another alternative, the salts, additives and liquids may be simultaneously combined. The liquid phase may additionally contain setting reaction or hydroxyapatite crystal growth modifiers such as a proteoglycan (e.g., hyaluronic acid), a protein (e.g., serum albumin), a carbohydrate (e.g., granular sugar), a synthetic material (e.g., polyethylene glycol), certain other ionic agents, and the like. Many additional representative examples of such materials may be found in U.S. Patent Nos. Re. 33,161 and Re. 33,221.

The tetracalcium phosphate component may be produced by any of various suitable methods. In one such method, the tetracalcium phosphate is produced by a solid state reaction catalyzed by high temperature treatment (e.g., 1500°C to 1700°C) of an equimolar mixture of dicalcium phosphate and calcium carbonate. The tetracalcium phosphate thus produced is then mixed with a desired amount of another sparingly soluble calcium phosphate salt selected from the group recited above. Alternatively, a mixture of α-$Ca_3(PO_4)_2$ and tetracalcium phosphate may be produced by calcining a hydroxyapatite preparation having a molar ratio of Ca/P of between 1.5 and 1.8 at 1150°C to 1450°C under reduced pressure. This mixture is further adjusted by the addition of a suitable sparingly soluble calcium phosphate salt in order to provide the desired setting properties and consistency of the hydroxyapatite formulation produced by precipitation upon mixing with the augmented liquid phase containing a bioactive or a biocompatible additive. The calcium phosphate salts and their combinations are preferably produced and stored under substantially

anhydrous conditions in order to precipitate hydroxyapatite formulations of consistent and superior quality upon addition of the liquid phase.

The following embodiments include methods of producing formulations comprising the calcium phosphate salt combinations mentioned above and various bioactive or biocompatible additives, formulations thus obtained, and uses therefor. In the discussion of each embodiment, representative bioactive or biocompatible substances intended for certain medical applications are described. A person of ordinary skill in the art will be able to produce such materials as described in these embodiments and will also be able (where appropriate or desirable) to substitute or add bioactive or biocompatible substances with properties similar to the ones cited in any particular embodiment. Also, while the foregoing discussion relates to calcium phosphate salt combinations such as those described by Brown and Chow, the technology herein is not so limited. The embodiments, features and aspects of the present invention can be applied to other calcium phosphate salt combinations. Further, the characteristics of the biocompatible hydroxyapatite formulations allow precipitation in vivo, ex vivo, or partially ex vivo and partially in vivo. Also, the hydroxyapatite may be resorbed or degraded in vivo.

1. Incorporation of Growth Factors For Wound Healing And Soft Tissue Repair

A first embodiment is directed to wound healing and soft tissue repair, and addresses a major need which exists for materials that can be applied to wounded soft tissues (e.g., skin, muscle, fascia, gum, periodontium, etc.) in order to cause or promote (as distinct from support) healing of the damaged tissue. Various biological factors have the potential for inducing growth of desired cells or formation of such cells from uncommitted precursor cells in the area of the damaged site. Systemic application of such materials would require substantial quantities, making such an approach both cost prohibitive and

impractical due to limitations on the availability of the materials. Even in cases where such problems have been solved through the advent of recombinant DNA technology, other activities often displayed by such factors and contaminants in preparations of these materials may produce undesirable side effects when such high amounts are administered systemically in order to achieve adequate concentration at a particular site. Local applications of such materials to date involve either coating of a biodegradable membrane covering type material or multiple applications over extended time periods. Such approaches are inefficient, fraught with risks of infection, and cumbersome.

A first embodiment of the present invention, which is directed to this medical application of augmentation or guided tissue regeneration, relates to a biocompatible hydroxyapatite formulation that incorporates one or more growth factors. The growth factor may be selected from the group consisting of epidermal growth factor (EGF), transforming growth factor alpha (TGF-α), transforming growth factor beta (TGF-β), vaccinia growth factor (VGF), acidic or basic fibroblast growth factor (FGF), insulin-like growth factor (IGF) (e.g., IGF-I and IGF-II), platelet-derived growth factor (PDGF), cartilage-derived growth factor (CDGF), interleukin-2, nerve cell growth factor (NCGF), hemopoietic cell growth factor (HCGF), lymphocyte growth factor (LGF), bone morphogenic protein (BMP) and other members of the growing family of wound healing factors. Further, the hemopoietic cell growth factor may be selected from the group consisting of interleukin-3, granulocyte-macrophage colony stimulating factor, angiogenesis factors, macrophage colony stimulating factor, granulocyte colony stimulating factor and erythropoietin. Also, the lymphocyte growth factor may be selected from the group consisting of B cell growth factor, T cell growth factor, interleukin-4, interleukin-5, and interleukin-6. The formulation preferably displays mechanical properties of a paste, a hydrogel, a film, or the like. The formulation preferably releases less than about 20% of the

growth factor in about 1 day and greater than about 90% of the growth factor in about 30 days. More preferably, the release rate equals less than 20% in about 2 days and greater than 90% in about 5 days. The amount of the growth factor is preferably from about 0.1 μg to about 10 μg per cubic centimeter volume of the formulation, and is more preferably in the range of from about 3μg to about 6μg per cubic centimeter volume.

The biocompatible hydroxyapatite formulation may be prepared according to the following steps. In a first step, a base combination of calcium phosphate salts is prepared. This step is preferably accomplished by performing the following procedure. One or more calcium phosphate salt combinations are prepared as described above. Then, a liquid phase is formed by supplementing a liquid, such as sterile water, saline or a physiologically compatible buffer with a crystal growth modifier, such as hyaluronic acid, non-cross-linked collagen, ethylene glycol, polyethylene glycol, glycerin, polylysine, or the like. The crystal growth modifier permits changes to the setting time and allows achievement of a desirable consistency, which depends on the method of administration. Next, the resulting liquid phase is added to the one or more salt combinations, respectively, preferably at a weight-to-weight solid-to-liquid ratio of between about 1:1 and about 5:1. The solid-to-liquid ratio is more preferably between about 1.5:1 and about 4:1, and most preferably between about 2.5:1 and about 3.5:1. Respective hydroxyapatite samples are precipitated from these mixtures, which are each evaluated for its setting property as well as its consistency. Each respective sample then, is allowed to harden to a sheet, each sheet preferably having a thickness ranging between about 1 mm and about 7 mm. More preferably, the thickness ranges from about 3mm to about 5mm. Pieces of the respective hardened materials are implanted subcutaneously and intramuscularly into test animals, e.g., rats, guinea pigs, rabbits or pigs. A base hydroxyapatite formulation, which permits the desired release rates, is then

selected. The base combination of calcium phosphate salts is that combination which can precipitate this base hydroxyapatite formulation.

In a second step, a liquid phase is augmented by the addition of a selected growth factor. This step is preferably accomplished according to the following procedure. First, a growth factor is selected, preferably from those already described. EGF, TGF-a, TGF-β or VGF, for example, may be produced by recombinant DNA technology from a transformed host cell substrate (e.g., mammalian, microbial, or insect cell culture), by chemical synthesis, or from cultured cell sources that elaborate these materials. Purified preparations are assayed in cell culture systems to determine the potency to promote cell growth and the specificity for binding to the receptor (EGF^R), and are stored as lyophilized powders. Other wound healing factors may be produced by recombinant or synthetic means, and are characterized for potency and specificity using cell culture stimulation assays and specific receptor binding assays, respectively. One or more varying amounts of the selected growth factor then are added to the supplemented liquid phase described in the above first step to provide one or more augmented liquid phases, respectively, having a range of final concentrations of the selected growth factor. Next, the respective augmented liquid phases are each mixed with the above-described base salt combination to precipitate respective augmented hydroxyapatite samples. Preferably, this mixing results in a solid-to-liquid weight ratio between about 1:1 and about 5:1 (more preferably between 1.5:1 and 4:1; most preferably between 2.5:1 and 3.5:1). These respective augmented hydroxyapatite samples then are allowed to harden in sheets having thicknesses preferably ranging from about 1 mm to about 7 mm (more preferably between 3mm and 5mm). Alternatively, the samples may be allowed to harden into test blocks. Test portions of the respective hardened samples are then immersed in water, saline, or other physiological fluids, such as serum or plasma, under sterile conditions at a

temperature from about 25°C to about 42°C and the respective test portions are gently agitated. The test portions from the liquid medium are then tested at various times, by immunological techniques, for amounts of growth factor released. A release kinetics diagram may be plotted from these testing results. Formulations which display about 30% or less release within about 5 days are chosen for subsequent in vivo testing. The chosen formulations are implanted subcutaneously and intramuscularly at vascularized sites in test animals. Release rates of the growth factor in the respective samples are determined by collecting serum samples as well as by collecting, via aspiration, local fluid surrounding the implant. Then a final augmented hydroxyapatite formulation is selected, which permits the aforementioned desired release rates. The augmented liquid phase is that which permits precipitation of this final augmented hydroxyapatite formulation.

In a third step, the augmented liquid phase is mixed with the base combination of calcium phosphate salts. And, in a fourth step, the biocompatible hydroxyapatite formulation is precipitated from this mixture.

2. Incorporation of Immunogens

The ability to incorporate an immunogen, e.g. a purified protein, glycoprotein, lipoprotein or the like, into a biocompatible implant that resorbs and releases the immunogen over several weeks has several advantages over known technology. These include, without limitation, avoiding the need for multiple booster injections that require multiple visits to the clinic by the subject, and enabling the elicitation of a more potent immune response with less immunogen material than is required when such immunogens are admixed with various adjuvants and administered by various conventional methods. Additionally, the implant may be formulated to produce low grade local inflammatory response, thereby enhancing the potency of the immunogen. A slow release approach may also enable immunization against materials that are

moderately toxic and thus not amenable to the development of an effective immune response by traditional immunization protocols.

The second embodiment of the present invention thus relates to a biocompatible hydroxyapatite formulation which incorporates an immunogen. For instance, the immunogen may be selected from the group consisting of viral antigens, bacterial antigens, fungal antigens, and parasitic antigens. The immunogen may also be any malignancy-specific marker including without limitation, tumor antigens, peptide fragments of tumor antigens, and metastatic-specific antigens. The immunogen may be a subunit vaccine. The immunogen may be incorporated in a vaccine, which can be active or passive. Also, the vaccine may be a synthetic vaccine which can be organically made or recombinantly made. Some specific examples of envisioned immunogens include HBV envelope antigen; HIV gp120/gp160/p41; recombinant or purified protein immunogens for vaccination against mumps, measles, rubella or small pox (vaccinia); and the like. Preferably, the formulation displays mechanical properties of a granule or plug. Preferably, the formulation releases about 20% or less of the immunogen in about 1 day and 90% or greater in about 30 days. More preferably, the release rates are 20% or less in about 2 days and 90% or more in about 5 days. The amount of the immunogen in the formulation is preferably between about 50 μg to about 500 μg per cubic centimeter packed volume. This amount is more preferably between about 100 μg to about 400 μg per cubic centimeter, and most preferably between about 150 μg to about 300 μg per centimeter.

A formulation according to this embodiment is preferably prepared according to the following steps. In a first step, a base combination of calcium phosphate salts is prepared. This first step is preferably accomplished according to the following procedure. First, one or more combinations of sparingly soluble calcium phosphate salts is prepared as previously described.

Next, a liquid phase is formed as previously described to have a solid-to-liquid ratio as previously described. In several following sub-steps, the liquid phases are mixed with the salt combinations to precipitate hydroxyapatite formulations which are each tested as described in connection with the first embodiment. Formulations that provide the desired resorption rates indicated above are selected. These formulations should permit the desired release rates of the immunogen.

In a second step, a liquid phase is augmented by the addition of a selected immunogen. This step is preferably accomplished according to the following procedure. First, an immunogen is selected. The immunogen may be any of those already described. Next, one or more varying amounts of the selected immunogen are added to the liquid phase described in the above first step to provide one or more augmented liquid phases, respectively, having a range of final densities of the selected immunogen. The respective augmented liquid phases then are each mixed with the above-described base salt combination to precipitate respective augmented hydroxyapatite samples. In subsequent steps, these samples are tested as described in connection with the first embodiment. A final augmented hydroxyapatite formulation is selected which permits the desired release rates. The augmented liquid phase for the second embodiment is that which permits precipitation of this selected final augmented hydroxyapatite formulation.

In a third step the augmented liquid phase is mixed with the base combination of calcium phosphate salts. And, in a fourth step, the biocompatible hydroxyapatite formulation is precipitate from this mixture.

3. Incorporation of Genes

A third embodiment of the present invention relates to gene therapy. Recent advances in the understanding of genetic bases for diseases and the ability to manipulate functional genes are revolutionizing the field of

medicine. The genetic material of humans and other animals, which is comprised of deoxyribonucleic acid (DNA), is amenable to direct transfer into recipient cells across species barriers. As early as twenty years ago, a method of transfer of DNA molecules to mammalian (including human) cells in tissue culture used a method of complexing DNA with calcium phosphate and applying this complex to the intended recipient cells. This resulted in the recipient cells, albeit at a very low frequency, taking up the calcium phosphate-DNA complex and allowing some of the DNA to be incorporated into the chromosomal material, thereby allowing the product encoded by the DNA to be produced in the recipient cells.

A major obstacle in delivering genes in the form of naked DNA into intact organisms (animals or humans) is the susceptibility of DNA to degrading enzymes in bodily fluids and the propensity of DNA delivered at target tissue sites, such as muscle tissue, to be washed away prior to the ability of the exposed cells to take up a reasonable amount. Some attempts at solving these problems have involved the attachment of genetic sequences derived from certain viruses whereby the desired piece of DNA, even if taken up by only a few cells, would be sufficient since the attached viral sequences would allow reproduction of the piece of DNA in the recipient cells. It was expected that progenies of the recipient cells and cell-to-cell transfer would amplify the delivered DNA dose in the animal (or human) in order to lead to a desired clinical outcome. A major safety concern, however, arises from the use of virus-derived sequences to render the desired genes into virus-like self reproducing elements. The concern is significant since most of the effective viral sequences that can provide the desired reproductive capability originate from highly infectious viruses including certain viruses known to cause deadly diseases, such as AIDS and cancer.

In the third embodiment of the present invention, a biocompatible hydroxyapatite formulation is provided for gene therapy, wherein the formulation incorporates a genetic material. The genetic material may comprise nucleic acids such as DNA or RNA (e.g., antisense), proteins or modified proteins (e.g., enzymes, transcription factors or translation factors), or cells that express a desired protein or nucleic acid. The genetic material (e.g. DNA) may be complexed in an ordered or random fashion with the hydroxyapatite. Cells physically adjacent to such complexes are allowed to take up the complex and hence the gene. Preferably, the formulation of the third embodiment incorporates purified DNA molecules (e.g., concatenated, circular or linear form) representing a coding sequence operably linked to regulatory and promoter sequences that allow favorable expression of a desired gene product in one or more types of mammalian cells. The formulation preferably displays mechanical properties of a soft paste or a slurry, and should deliver 20% or less of the genetic material about 1 day and 90% or greater in about 30 days. More preferably, the delivery rates are 20% or less in about 2 days and 90% or greater in about 5 days. The level of DNA is preferably in the range of from about 10 μg to about 100 μg per cubic centimeter of the delivery vehicle produced according to this embodiment. More preferably, the level of DNA is preferably between 10 μg and 50 μg.

The biocompatible hydroxyapatite formulation of this embodiment is preferably prepared according to the following steps. In a first step, a base combination of calcium phosphate salts is prepared. This may be accomplished according to the following procedure, which is similar to that of previous embodiments. First, one or more combinations of calcium phosphate salts are prepared as previously described. Then, a liquid phase is prepared, also as previously described. Mixtures of these components, preferably having solid-to-liquid ratios as discussed in connection with previously described embodiments,

liquid ratios as discussed in connection with previously described embodiments, are tested as discussed above.

In a second step, a liquid phase is augmented by addition of the desired genetic material. This step may be accomplished according to the following procedure. First, a desired gene ("coding sequence") may be selected which encodes the product intended to be expressed in the host. The gene may be cloned, propagated in a microbial host, and edited for expression either in a broad variety of recipient host cells or in a tissue-specific manner. Required regulatory elements necessary for expression include general or tissue-specific promoter sequences, splice signals, polyadenylation signals and enhancer sequences. The genetic construct to be incorporated in the hydroxyapatite formulation may be derived by combining the coding sequence with an appropriate set of regulatory elements in an operable linkage. The construct may then be propagated in a microbial host in order to avoid the risks of contaminating potentially hazardous genetic sequences from a mammalian cell or a human cell-directed virus. Preferably, all parts of the genetic construct are cloned from normal human cellular genetic material and then modified or edited and propagated in microbial host systems. Alternatively, where a viral or oncogene sequence is desired to be expressed (e.g., for immunotherapy purposes against certain cancers, AIDS, and the like), the coding sequence may be derived from the appropriate virus or tumor cells. In certain cases, the coding sequence may be created by deleting "introns" from the original chromosome-derived material or through the creation of a DNA copy of the functional messenger RNA by a process called reverse transcription. In other cases, if the amino acid sequence of a desired polypeptide or protein product is known, the coding sequence for such a protein or altered forms of such a protein with predetermined changes may be produced by chemical gene synthesis. In these situations, where the coding sequence is modified or synthetically produced,

editing of the coding sequence may involve predetermined addition of new splice signals, transcription/translation start sites, or the like, in order to facilitate the production of the product encoded by the coding sequence in the recipient cells. Purified DNA for the biocompatible hydroxyapatite formulation of this embodiment preferably comprises the functional gene construct produced as described herein in a linear or circular form, in a single copy or tandemly linked multiple copy form, or in a composite of these forms.

Various amounts of the desired gene, e.g., purified DNA, are then dissolved in a liquid phases of the type described in connection with previous embodiments. It is envisioned that the desired gene may be encompassed in a cell, which may be a recombinant cell. The cell may be a myeloid-derived cell or a lymphoid-derived cell. The cell may express a recombinant product including, without limitation, those selected from the group consisting of insulin, nucleic acid, viral antigens, bacterial antigens, fungal antigens, parasitic antigens, cytokines, growth factors, hormones, cell surface proteins, and enzymes. It is also envisioned that a protein or a nucleic acid may be directly added to the liquid phase.

Mixing of the genetic material with the liquid phase preferably results in the previously described solid-to-liquid weight ratios. These augmented liquid phases are mixed with the base salt combination to provide augmented hydroxyapatite samples which are tested as described previously. A final augmented hydroxyapatite formulation is selected, which permits the desired delivery rates. The augmented liquid phase is that which leads to this final augmented hydroxyapatite test formulation.

In a third step, the augmented liquid phase is mixed with the base combination of calcium phosphate salts. And, in a fourth step, the biocompatible hydroxyapatite formulation is precipitated from this mixture.

4. Extended Or Controlled Delivery Of Pharmaceutical Agents

Traditional pharmaceutical agents typically comprise small chemical molecules that are cleared from the circulatory system relatively rapidly. An undesirable consequence of this trait is the resulting need to administer a compound containing the desired agent to the patient multiple times over several days or weeks. Also, each administration involves the use of a large amount of the compound so that, after the initial dilution, enough compound is present in the circulatory system to cause a desired therapeutic effect. Current approaches to address these problems have primarily involved mechanical methods, e.g., continuous intravenous infusion or the use of implantable pumps. An implantable substance that contains the desired agent and degrades over different time periods, thereby releasing the agent and biocompatible ions such as calcium and phosphate, would improve upon known approaches. Among other advantages, such an approach reduces the complexity of conventional administration of pharmaceutical agents, which is more expensive, fraught with risks of infection, and subject to reduced patient compliance. An implantable substance according to this embodiment would also lower the total amount of the pharmaceutical agent needed over the total course of treatment, and would avoid potential side effects caused by high concentrations of the pharmaceutical agent following each administration.

Accordingly, the fourth embodiment relates to a biocompatible hydroxyapatite formulation that incorporates a pharmaceutical agent. Preferably, the agent is a biocide selected from the group consisting of anti-neoplastic agents, anti-bacterial agents, and anti-parasitic agents. An anti-neoplastic agent may be selected from the group consisting of cyclophosphamides, alkylating agents, purine analogs, pyrimidine analogs, vinca and vinca-like alkaloids, etoposides and etoposide-like drugs, antibiotics, corticosteroids, nitrosoureas, antimetabolites, platinum-based cytotoxic drugs, hormonal antagonists, antiestrogens, tamoxifen, doxorubicin, L-asparaginase,

dacarbazine, amsacrine, procarbazine, hexamethylmelamine and mitoxantrone. An anti-bacterial agent may comprise a heavy metal, an antibiotic, or another anti-bacterial agent. The pharmaceutical agent may also be an inflammatory agent, an analgesic, or a chemotherapeutic substance. Other pharmaceutical agents envisioned are known to those having ordinary skill in the art. The biocompatible hydroxyapatite formulation should display the mechanical properties of granules or a soft paste. The formulation should be capable of releasing 20% or less of the agent within 1 day and 90% or greater with about 30 days. More preferably, the release rates are 20% or less within about 2 days and 90% or greater within about 10 days. The level of the agent preferably comprises from about 10% to about 50% of the total dosage conventionally prescribed for a full course of a specific treatment.

The biocompatible hydroxyapatite formulation according to the fourth embodiment is preferably produced by the following steps. In a first step, a base combination of calcium phosphate salts is prepared by following a procedure similar to that in the previous embodiments.

In a second step, a liquid phase is augmented by adding a desired pharmaceutical agent. This second step is preferably accomplished by the following procedure. The desired agent is selected from among the agents already described. Then, one or more liquid phases, which are prepared as in previous embodiments, are augmented by adding varying amounts of the pharmaceutical agent. Each of the augmented liquid phases then are respectively mixed with the base salt combination preferably at a solid-to-liquid ratio as previously described, to form an augmented formulation. Test samples of the respective augmented formulations (having varying amounts of the pharmaceutical compound) are implanted subcutaneously, intramuscularly or intraperitoneally into test animals and characteristics such as serum levels of the pharmaceutical agent compound and resorption rate of the implanted formulation

are monitored. A final augmented formulation is selected which displays mechanical properties of granules or a paste, which permits the desired release rates of the agent, and which has a level of the agent of about 10% to about 50% of the total dosage currently prescribed for a full course of treatment. The augmented liquid phase is that which leads to this final augmented test formulation.

In a third step, the base combination of calcium phosphate salts is mixed with the augmented liquid phase. And, in a fourth step, the biocompatible hydroxyapatite formulation is precipitated from the mixture of the third step.

According to one example of this embodiment, antibiotics and anti-inflammatory compounds are incorporated into a hydroxyapatite formulation, wherein a final augmented formulation is selected that provides a suitable dosage for about 7 to about 14 days. In another example, a chemotherapeutic substance (or a combination thereof with other substances) is incorporated into a hydroxyapatite formulation to produce an augmented formulation capable of delivering the chemotherapeutic substance over a period of about 20 to about 30 days.

5. Extended Or Controlled Delivery Of Hormones

Small peptide hormones or their derivatives are believed to exert their physiological action on specific target cells by binding to specific membrane receptors on such cells. As in the case of small chemical molecules that are incorporated in conventional pharmaceutical agents, the small peptides have only a limited circulating half-life. This characteristic can be significantly improved by incorporating such molecules in a biodegradable hydroxyapatite material.

Thus, according to a fifth embodiment of the present invention, a biocompatible hydroxyapatite formulation is provided as a delivery vehicle for

hormones including peptide hormones and hormone-like agents. Preferably, the formulation comprises a hormone or peptide factor such as a regulatory-type hormone selected from the group consisting of insulin, atrial natriuretic factor (ANF), calcitonin, vasopressin, relaxin and the like. The hormone may also be a sex hormone selected from the group consisting of estrogenic hormones, progestational hormones, androgenic hormones and any active derivative of these. The formulation should display mechanical properties of a paste suitable for subcutaneous placement and percutaneous application. Preferably, the formulation is capable of delivering the hormone at a rate of about 20% or less in about 1 day and about 90% or greater in about 30 days. More preferably, the delivery rates are 20% or less in about 2 days and 90% or greater in about 7 days. The concentration of the active material in the formulation is preferably from about 10% to about 50% (per cubic centimeter of the administered formulation) of the conventional recommended cumulative dosage for a conventional treatment period of about 5 to about 30 days.

The biocompatible hydroxyapatite formulation may be accomplished by the following steps. In a first step, a base combination of calcium phosphate salts is prepared preferably by following the procedure described in the fourth embodiment (i.e., the embodiment incorporating a pharmaceutical agent) or steps similar thereto. In a second step, a liquid phase, such as that described in the previous embodiments, is augmented by adding the desired hormone (or peptide factor or hormone-like agent). This is preferably accomplished by following the procedure describe in the fourth embodiment. In a third step, the base salt combination is mixed with the augmented liquid phase. And, in a fourth step, the biocompatible hydroxyapatite formulation is precipitated from the mixture of the third step.

6. Bone Replacement, Repair and Regeneration

Bone is unique in its ability to fully regenerate among the complex tissues in higher organisms such as vertebrates. Due to the complex nature of bone tissue and the varied functions performed by the skeleton at different locations within the body, different approaches for intervention may provide greater therapeutic benefits in different locations. The respective roles played by the cellular component, the matrix component, and the interaction between these two phases of bone, contribute to the regenerative process and the functioning of the tissue. Certain proteins that are unique to bone have been identified with respect to their structural and mineral binding properties. Thus, a sixth embodiment of the present invention relates to bone replacement, repair and regeneration.

One aspect of this embodiment deals with osteoconductive principles. A biocompatible hydroxyapatite formulation which represents the major mineral component of bone in the presence of the major structural and mineral binding proteins found in bone, may be provided which has utility as a filler of bone voids with a matrix resembling that of normal bone. Such a formulation is compatible with bone and capable of undergoing resorption to be replaced by new bone that remodels along natural processes. This novel formulation is useful in filling various damaged sites and is also useful in filling gaps between prosthetic devices and surrounding bony tissue, thus providing a better attachment for orthopedic, periodontal and dental applications. The formulation is also useful as an extender of autografts or allografts where the amounts normally available are not sufficient.

Thus, according to this aspect, a biocompatible hydroxyapatite formulation is provided which is capable of supporting bone growth. Preferably, a formulation according to this aspect contains one or more adhesive agents. The adhesive agent may be chosen from the group consisting of integrins, extracellular matrix proteins, leukocyte adhesion proteins, collagen,

albumins, bone proteins, osteonectins, cell surface receptor proteins, bone *gla* protein, and matrix *gla* protein. The biocompatible hydroxyapatite formulation should display mechanical properties of a paste that hardens into an implant. The implant preferably has a compressive strength greater than 10 MPa, which is more preferably greater than about 50 MPa, and most preferably greater than about 100 MPa. The implant should be about 45% porous with an average pore size of from about 15 microns to about 30 microns. More preferably the average pore size is from about 20 microns to about 25 microns. Most preferably, the average pore size equals about 23 microns. The maximum pore size should be less than 100 microns, preferably less than 50 microns. The formulation is preferably resorbable within about 60 days to about 2 years. More preferably, the formulation is resorbable within about 60 to about 90 days. The amount of the osteogenic protein should be from about 10% to about 40% of the total weight of the formulation, and the relative amount of the proteins to each other (if more than one is incorporated) should be similar to that found in the bony tissues at the site of application. The biocompatible hydroxyapatite formulation may additionally contain (a) an antibiotic substance that is locally released from the formulation during the repair process and prevents infections that could hinder regeneration; or (b) a hormone, e.g., calcitonin, that may be locally released during the repair process to inhibit bone loss due to an underlying metabolic disease such as osteoporosis.

The augmented hydroxyapatite formulation of this aspect of the sixth embodiment is preferably formed according to the following method. In a first step, a base combination of calcium phosphate salts is prepared. This may be accomplished according to a procedure similar to that of previous embodiments.

In a second step, a liquid phase is augmented by adding an osteogenic adhesive-type protein. This may be accomplished by the following

procedure. First, one or more proteins are selected from those already described. For example, human bone *gla* protein, human matrix *gla* protein and human osteonectin may be produced by recombinant means and purified. Then, a liquid phase is formed as described previously. The liquid phase may be supplemented (or additionally supplemented) with granular sugar, such that upon mixing with the base salt combination the final formulation will contain up to about 20% by weight of sugar. The selected proteins are then uniformly dissolved in the supplemented liquid phase to form an augmented liquid phase. In a third step, the base salt combination is mixed with the augmented liquid phase. In a fourth step, the biocompatible hydroxyapatite formulation is precipitated from the mixture of the third step.

The paste thus formed is preferably allowed to harden under pressure (e.g., from about 40,000 psi to about 80,000 psi) in vitro in desirable mold conformations for a period of about 12 hours to about 48 hours. The hardened formulation is then subjected to treatment with warm water at temperatures in the range from about 50°C to about 70°C, more preferably about 50°-55°C. This treatment should last for about 4 to about 10 hours, more preferably from 4 to 5 hours, to remove most of the granular sugar, thus creating a highly porous material. The material may then be implanted into a body, for example, at a selected bone void.

Another aspect of the sixth embodiment deals with osteoinduction. Hydroxyapatite implants, including the porous forms, frequently allow ingrowth of newly developed bony tissue at bone defect sites. As a result, the hydroxyapatite implant is incorporated into the newly formed bone which remodels naturally. A hydroxyapatite implant that can actively effect the differentiation process that causes the pluripotent stem cells at the site of a trauma to follow the path of bone formation - "osteogenesis" - will provide improved therapeutic benefits. A group of factors, generally called osteogenic

factors, bone morphogenic protein, or chondrogenic factors, have been shown to mediate such phenomenon in animals and humans.

Thus, this aspect of the sixth embodiment of the present invention provides a biocompatible hydroxyapatite formulation, which may cause active ingrowth of bone into implants. Preferably, the formulation is capable of causing induction of new bone formation and comprises one or more growth factors selected from the group consisting of osteogenic factors, bone morphogenic, proteins, and chondrogenic factors. These growth factors may be included either alone or in combination with one or more adhesive agents, which may be selected from the group consisting of integrins, extracellular matrix proteins, leukocyte adhesion proteins, collagen, albumins, bone proteins, osteonectins, cell surface receptor proteins, bone *gla* protein, and matrix *gla* protein. The formulation should display mechanical properties of a paste that hardens into an implant having a tensile strength of at least 20 MPa. More preferably, the tensile strength is greater than 60 MPa, and most preferably greater than about 70MPa. The formulation is preferably porous with average and maximum pore sizes as described above. The level of the active growth factor should be in the range of from about 10 μg to about 100 μg for every cubic centimeter of a delivery vehicle containing the selected growth factor alone, or of a delivery vehicle containing up to about 40 wt% of the one or more adhesive agents.

The final formulation may additionally contain a heparanase. This term is generically intended to mean an enzyme that either degrades heparin sulphate or causes the release of molecules in vivo that bind to heparin or heparin sulphate. The final formulation may also contain an antibiotic substance.

The formulation of this aspect of the sixth embodiment may be prepared according to the following method. In a first step, a base combination

of sparingly soluble calcium phosphate salts is prepared as before. In a second step, a liquid phase is augmented by adding the appropriate one or more growth factors and the desired adhesive agents. Various suitable factors, including those already mentioned as examples, have been described in the art along with recombinant means of production either in genetically engineered mammalian cells or microbial host cells. In a third step, the augmented liquid phase is mixed with the base salt combination. Preferably, this mixture has a solid-to-liquid ratio as described previously. In a fourth step, the biocompatible hydroxyapatite formulation is precipitated from the mixture of the third step. The formulation preferably has the above described characteristics. The final formulation may be hardened ex vivo or rapidly in vivo when applied in an open fracture as an implant or a grouting material in conjunction with other implants.

Still another aspect of the sixth embodiment focuses on bone regeneration. Bone defects at different skeletal sites may require different balances between bone regeneration and short term biomechanical support. The formulations described in the previous aspect of the sixth embodiment can provide modest mechanical support and allow efficient bone ingrowth such that the implant is rapidly incorporated into newly formed bone for long term mechanical support. Yet, these formulations also provide reasonable stress bearing capability within a short time post-operatively by virtue of their biomechanical strength. In other applications, where short term load bearing is not an important factor (e.g., spinal fixation, radial and ulnar non-union fracture, and the like), there is a need to be able to cause rapid formation of new bone for long term benefit with short term support being provided by other fixation devices.

Thus, according to this aspect of the sixth embodiment of the present invention, a biocompatible hydroxyapatite formulation is provided which is capable of actively inducing bone repair in small defects. The formulation

preferably comprises a growth factor selected from the group consisting of osteogenic factors, bone morphogenic proteins, and chondrogenic factors. The formulation may also comprise one or more adhesive agents, which may be selected from the group consisting of integrins, extracellular matrix proteins, leukocyte adhesion proteins, collagen, albumins, bone proteins, osteonectins, cell surface receptor proteins, bone *gla* protein, and matrix *gla* protein. The formulation should be in the form of a soft paste or slurry that can be applied percutaneously. Preferably, the formulation is characterized by the resorbability described in connection with the previous aspects and preferably contains between about 50 μg and about 500 μg of the selected growth factor for every cubic centimeter of the formulation. If incorporated, the adhesive agent should be at a level of up to 40 wt% of the final formulation.

The formulation additionally may contain heparanase, which is generically intended to mean an enzyme that either degrades heparin sulphate or induces the release of molecules in vivo which bind to heparin or heparin sulphate. The formulation may additionally contain an antibiotic substance. Compared to the formulation described in the previous aspects of this embodiment, this formulation is not intended to have significant mechanical strength and is intended to provide a more rapid, local delivery of growth factors for a shorter duration.

The formulation of this aspect of the sixth embodiment may be prepared according to the following method. In a first step, a base combination of sparingly soluble calcium phosphate salts is prepared as before. In a second step, a liquid phase is augmented by adding the appropriate one or more growth factors and the desired adhesive agents. In a third step, the augmented liquid phase is mixed with the base salt combination. In a fourth step, the biocompatible hydroxyapatite formulation is precipitated from the mixture of the third step. The formulation preferably has the above-mentioned characteristics.

Also, this mixture should have a solid-to-liquid ratio as previously described. Preferably, the final formulation is applied percutaneously and hardens in vivo.

Examples of this and the previous aspects of this embodiment include: (a) a formulation that incorporates a homodimer of BMP-2 produced in Chinese hamster ovary (CHO) cells; (b) a formulation that uses molecules designated COP5 or COP7 or Vg1 produced in E coli cells; and (c) a formulation that incorporates a heterodimer of BMP-4 and BMP-5 produced in a genetically engineered mammalian host cell.

7. Enhancement Of Electrical Or Electromagnetic Stimulation Of Bone Growth

A seventh embodiment of the present invention relates to the enhancement of osteogenesis around and into hydroxyapatite implants in response to electromagnetic stimulation. Electromagnetic stimulation has been reported to be effective in stimulating bone healing and bone ingrowth into hydroxyapatite implants when the implants have an average pore size preferably in the range of from about 15 microns to about 30 microns (more preferably between 20 microns and 25 microns; most preferably equal to about 23 microns). The incorporation of paramagnetic, diamagnetic, conductive, and insulating material into an implant can intensify or attenuate the electrical and magnetic fields proximal to the implant when it is subjected to electromagnetic stimulation. This improved formulation can increase the rate of bone healing and reduce the field intensity required for electromagnetic stimulation. This embodiment of the present invention relates to an augmented hydroxyapatite formulation with altered magnetic and conductive properties which enhance the effects of electromagnetic field-induced bone repair and bone formation.

Thus, the seventh embodiment of the present invention provides a biocompatible hydroxyapatite formulation, which incorporates an electrical stimulus enhancer. The stimulus enhancer may be one or more of a

paramagnetic material, a diamagnetic material, a conductive material, or an insulator. The paramagnetic material may be selected from the roup consisting of iron, iron ammonium alum, uranium, platinum, and aluminum. The diamagnetic material may be selected from the group consisting of bismuth, mercury, silver, carbon (diamond), lead, and copper. The conductive material may be selected from the group consisting of silver, copper, aluminum, and tungsten. And, the insulator may be selected from the group consisting of glass, lucite, mica, quartz, and polytetrafluoroethylene (PTFE). Preferably, the formulation is capable of causing new bone ingrowth and promoting osteogenesis in the tissue surrounding the implant. The formulation should display mechanical properties of a paste that hardens into an implant having a tensile strength greater than 20 MPa (more preferably greater than 60 MPa; most preferably greater than 70 MPa). The formulation should be porous with average and maximum pore sizes as described above. The formulation preferably has the resorbability already described (i.e., 60 days to 2 years; preferably 60-90 days).

The hydroxyapatite formulation of the seventh embodiment may be prepared according to the following method. In a first step, a base combination of sparingly soluble calcium phosphate salts is prepared as before. In a second step, a liquid phase is augmented by adding one or more electromagnetic enhancers selected from those already described. In the cases where the material is toxic, it can be encased in glass or PTFE. In a third step, the augmented liquid phase is mixed with the base salt combination. And, in a fourth step, the biocompatible hydroxyapatite formulation is precipitated from the mixture of the third step.

The hydroxyapatite formulation may be hardened in vitro and used as a bone replacement. In a treatment example, an implant formed from the formulation may be implanted in a human body. Next, the implant is

stimulated electrically or magnetically to induce bone growth within and around the implant.

8. Enhancement Of Electrical Or Electromagnetic Pulse In Local Release Of Materials

Several embodiments of this invention generally relate to the use of a hydroxyapatite formulation for the delivery of a bioreactive substance. Application of an electromagnetic field can stimulate bone and tissue growth and vascularization in and around a hydroxyapatite implant. Electromagnetically stimulating a hydroxyapatite implant or implanted hydroxyapatite formulation can also regulate the delivery of bioreactive substances. Electric fields have been shown to cause desorption of factors from a matrix which is exposed to the field. In addition to causing direct release, electromagnetic stimulation will also stimulate tissue ingrowth into the implant, thus increasing contact between the bioreactive substance and the tissue.

Thus, the eighth embodiment of the present invention relates to a biocompatible hydroxyapatite formulation which is subjected to electrical or electromagnetic stimulation after being inserted into a human or animal. The hydroxyapatite formulation is preferably prepared according to the following method. In a first step, a base combination of calcium phosphate salts is prepared as before. In a second step, a liquid phase is augmented by adding one or more bioreactive substances. The bioreactive substance can be any of the biocompatible additives described herein. In a third step, the augmented liquid phase is mixed with the base salt combination. In a fourth step, the biocompatible hydroxyapatite formulation is precipitated from the mixture of the third step. The precipitation can be in vivo. Alternately, the precipitation can be ex vivo. According to this alternative, an additional step is required in which the precipitated hydroxyapatite formulation is implanted into a human or animal. In a fifth step, the implant is stimulated electrically or electromagnetically to

induce bone and tissue growth and vascularization within and around the hydroxyapatite implant and to induce release of the bioreactive substance from the hydroxyapatite formulation.

9. Multicomponent, Layered Devices For Dual Tissue Interfacing

The use of implantable devices in guided tissue regeneration processes would be significantly more beneficial if such devices could be constructed to allow one surface to be compatible with one tissue type while another surface is compatible with a second tissue type. In periodontal defects, for example, the devices interface with both the soft tissue of the gum and the underlying bony structure. Similarly, in articular joints such as hip, knee, elbow and ankle joints, where rheumatoid arthritis or osteoarthritis has caused cartilage and bone damage, a desirable device should have one surface compatible with bone and another compatible with cartilage. It will be readily apparent to one of ordinary skill that other examples could be easily identified with application throughout the human body, as well as animals.

Thus, the ninth embodiment, which may be used for such dual tissue interface applications, provides a layered device having a first layer comprising a synthetic polymer (e.g., a glycolide/lactide/acrylic material selected for compatibility with soft tissues or cartilage) and a second layer of a biocompatible hydroxyapatite formulation which is compatible with bone. The second layer may be created by the application of a paste formed from a sparingly calcium phosphate salt mixture, such as those previously described. This embodiment further contemplates the incorporation of one class of pharmaceutically active or biocompatible material in the first layer to facilitate the regeneration of soft tissue or cartilage while the hydroxyapatite layer incorporates one or more biologically active or biocompatible substances, which facilitate bone repair as described in previous embodiments.

A significant advantage of the layered devices of this embodiment is that biologically or pharmaceutically active substances that negate the formation of selected tissues may be incorporated into one layer while other substances which encourage such formation can be incorporated into the other layer. This allows clear delineation of the dual tissue interface created in the guided tissue regeneration process. For example, in the case of articular joints, the synthetic polymer layer that is made to be compatible with cartilage may contain an anti-angiogenic factor to prevent migration of bone tissue into this layer from the bony tissue being regenerated at the hydroxyapatite layer. According to an example, the synthetic polymer layer is impregnated with Type II collagen and hyaluronic acid to allow better cartilage compatibility, while the hydroxyapatite layer is impregnated with Type I collagen, bone *gla* protein, osteonectin and the like, for the facilitation of bone regeneration.

10. Other Features and Aspects

The embodiments thus described are merely intended as examples of the present invention, which is not limited thereto. For example, as previously discussed, many of the embodiments described herein involve a process wherein a liquid phase is augmented with a biocompatible additive. Then, the augmented liquid phase is mixed with a base combination of calcium phosphate salts. However, it is envisioned that the components of the mixture, which precipitates hydroxyapatite, can be combined in different orders or simultaneously. Also, although various release rates are described, those having ordinary skill will recognize that the components of the hydroxyapatite formulations may be manipulated to achieve different release rates which may be more suitable for a given application.

According to a feature of the various embodiments of the present invention, the components of the mixture which precipitates a biocompatible hydroxyapatite formulation may be provided in a kit form. For example, a

package comprises a kit having three vials. A first vial preferably contains a measured amount of the base calcium phosphate salt combination. A second vial preferably contains the selected additive, which may be any of the additives already described (e.g., a growth factor). Depending upon the additive and desired treatment criteria, the additive may be in any of several forms. For example, a growth factor might be provided in a lyophilized state. The third vial contains the liquid phase. Alternately, the kit could contain two vials. In a first two-vial options, one vial would contain the calcium phosphate salts and the other would contain a mixture of the liquid phase and the additive. In a second two-vial option, one vial contains a combination of the additive and the salts, while the other vial contains the liquid phase.

According to an aspect of the kit feature, the contents of the vials may be combined to produce a paste of the biocompatible hydroxyapatite material. The resulting paste may be administered by itself in a surgical site or may be used to augment sutures, staples, membranes (resorbable or nonresorbable) or the like that are in widespread use for wound stabilization and closure. The paste may also be used to augment artificial skin and membranes used to cover major burn wounds.

According to another feature of the previous embodiments, pre-impregnated hardened sheets of a precipitated biocompatible hydroxyapatite formulation incorporating an additive may be provided in sterile form. Preferably, these sheets are capable of being cut into desired shapes and applied to cover wound sites or to pack deep wounds. Also, the sheets thus applied may be stabilized using known surgical closure techniques. Various processes of producing the sheets or similar structures are contemplated in this embodiment.

The embodiments described above may also incorporate an antibiotic substance that prevents infection of the augmented hydroxyapatite material. The antibiotic substance may also be released at the wound site in

order to prevent infection of the wound site while the material is in place. Some of the types of antibiotics which may be used include, without limitation, aminoglycosides, amphenicols (e.g., chloramphenicol), β-Lactams antibiotics, penicillins (e.g., ampicillin), peptide antibiotics, and tetracycline antibiotics (e.g., tetracycline).

According to another feature, two or more formulations are independently prepared. For example, an implant may be prepared ex vivo by mixing the salts, additives and liquid phase as described above to produce a paste. The paste may be allowed to harden into a component which is more slowly resorbable than a separately prepared formulation. This paste may be extruded through an appropriate aperture to produce granules of such a size that may be applied through a large gauge hypodermic needle or other acceptable injector mechanism. A second formulation may be freshly prepared at the time of administration (e.g., by adding a liquid phase containing approximately one third of the total intended dosage of the additive to the base salt combination at a ratio that produces a softer paste, which can harden in situ into a more rapidly biodegradable implant. Adequate amounts of the granules can be mixed uniformly with the base salt combination prior to adding the augmented liquid phase, such that the granules contribute about three times as much of the immunogen as contributed by the softer paste. Such a system comprising a paste with admixed granules and a freshly prepared paste provides a two-component system wherein the freshly prepared paste releases a first dose of the selected additive relatively rapidly and the admixture of paste and granules delivers booster amounts more slowly over an extended time period. According to an aspect of this feature, a layered composite may be produced wherein the central core contains a more slowly resorbable formulation that is surrounded by a more rapidly resorbable formulation.

According to still other features of these embodiments, the formulation may be administered intramuscularly, intravenously, subcutaneously or percutaneously (depending on the desired target tissue) where the additive (e.g., DNA) is to be delivered. The solid-to-liquid ratio and the amounts of supplements recited herein for the liquid phase are selected based on results of animal evaluations where various test formulations are administered, and the release of the additive and the biodegradation of hydroxyapatite are monitored.

It will be recognized by those having ordinary skill in the pertinent art that other variations and modifications to the specific examples disclosed herein can be easily accomplished without departing from the scope and spirit of the present invention. Accordingly, the present invention is only limited by the following claims.

We Claim:

1. A method for preparing a biocompatible hydroxyapatite formulation comprising the steps of:

a) preparing a base combination of calcium phosphate salts;

b) preparing a liquid phase;

c) providing a biocompatible additive;

d) combining the base combination of calcium phosphate salts, the liquid phase and the biocompatible additive to form a mixture; and

e) precipitating the biocompatible hydroxyapatite formulation from the mixture.

2. The method of claim 1 wherein the combining step comprises:

a) adding the biocompatible additive to the liquid phase to form an augmented liquid phase; and

b) mixing the augmented liquid phase with the base combination of calcium phosphate salts.

3. The method of claim 1 wherein the combining step comprises:

a) adding the biocompatible additive to the base combination of calcium phosphate salts to form an augmented combination of calcium phosphate salts; and

b) mixing the liquid phase with the augmented combination of calcium phosphate salts.

4. The method of claim 1 wherein the combining step comprises simultaneously combining the base combination of calcium phosphate salts, the biocompatible additive and the liquid phase.

5. The method of claim 1, wherein said precipitating step occurs in vivo.

6. The method of claim 1, wherein said precipitating step occurs ex vivo.

7. The method of claim 1, wherein said precipitating step occurs partially ex vivo and partially in vivo.

8. The method of claim 1, further comprising the step of permitting the precipitated biocompatible hydroxyapatite formulation to be resorbed or degraded in vivo.

9. The method of claim 1 wherein the base combination comprises two salts and wherein one of the two salts is tetracalcium phosphate and the other of the two salts is selected from the group consisting of $CaHPO_4 \cdot 2H_2O$, $CaHPO_4$, $Ca_8H_2(PO_4)_6 \cdot 5H_2O$, β-$Ca_3(PO_4)_2$, α-$Ca_3(PO_4)_2$, and modified $Ca_3(PO_4)_2$.

10. The method of claim 9 wherein the modified $Ca_3(PO_4)_2$ is tricalcium phosphate modified by protons or up to about 10 wt % magnesium.

11. The method of claim 1 wherein the biocompatible additive is a growth factor.

12. The method of claim 11 wherein the growth factor is selected from the group consisting of epidermal growth factors, transforming growth factor α, transforming growth factor-β, vaccinia growth factors, fibroblast growth factors, insulin-like growth factors, platelet-derived growth factors, cartilage-derived growth factors, interleukin-2, nerve cell growth factors, hemopoietic cell growth factors, lymphocyte growth factors, bone morphogenic proteins, osteogenic factors, chondrogenic factors.

13. The method of claim 12 wherein the hemopoietic cell growth factor is selected from the group consisting of interleukin-3, granulocyte-macrophage colony stimulating factor, angiogenesis factors, macrophage colony stimulating factor, granulocyte colony stimulating factor, and erythropoietin.

14. The method of claim 12 wherein the lymphocyte growth factor is selected from the group consisting of B cell growth factor, T cell growth factor, interleukin-4, interleukin-5, and interleukin-6.

15. The method of claim 1 wherein the biocompatible additive is an immunogen.

16. The method of claim 15 wherein the immunogen is selected from the group consisting of a viral antigen, a bacterial antigen, a fungal antigen, and a parasitic antigen.

17. The method of claim 15 wherein the immunogen is a malignancy-specific marker.

18. The method of claim 17 wherein the malignancy-specific marker is selected from the group consisting of tumor antigens, peptide fragments of tumor antigens, and metastatic-specific antigens.

19. The method of claim 15 wherein the immunogen is a subunit vaccine.

20. The method of claim 1 wherein the biocompatible additive is a vaccine.

21. The method of claim 20 wherein the vaccine comprises an antigen selected from the group consisting of a viral antigen, a bacterial antigen, a fungal antigen, and a parasitic antigen.

22. The method of claim 20 wherein the vaccine is a passive vaccine.

23. The method of claim 20 wherein the vaccine is an active vaccine.

24. The method of claim 20 wherein the vaccine is a synthetic vaccine.

25. The method of claim 24 wherein the synthetic vaccine is made by organic synthesis.

26. The method of claim 24 wherein the vaccine is made by recombinant techniques.

27. The method of claim 1 wherein the biocompatible additive is a nucleic acid.

28. The method of claim 1 wherein the biocompatible additive is a protein.

29. The method of claim 28 wherein the protein is selected from the group consisting of insulin, nucleic acid, viral antigens, bacterial antigens, fungal

antigens, parasitic antigens, cytokines, growth factors, hormones, cell surface proteins, and enzymes.

30. The method of claim 1 wherein the biocompatible additive is a cell comprising a gene.

31. The method of claim 30 wherein the cell is a recombinant cell.

32. The method of claim 30 wherein the cell is a myeloid-derived cell.

33. The method of claim 30 wherein the cell is a lymphoid-derived cell.

34. The method of claim 30 wherein the cell expresses a recombinant product.

35. The method of claim 34 wherein the recombinant product is selected from the group consisting of insulin, nucleic acid, viral antigens, bacterial antigens, fungal antigens, parasitic antigens, cytokines, growth factors, hormones, cell surface proteins, and enzymes.

36. The method of claim 1 wherein the biocompatible additive is a pharmaceutical agent.

37. The method of claim 36 wherein the pharmaceutical agent is selected from the group consisting of anti-neoplastic agents, anti-bacterial agents, anti-parasitic agents, and derivatives and combinations thereof.

38. The method of claim 37 wherein the anti-neoplastic agent is selected from the group consisting of cyclophosphamides, alkylating agents, purine analogs, pyrimidine analogs, vinca and vinca-like alkaloids, etoposides and etoposide-like drugs, antibiotics, corticosteroids, nitrosoureas, antimetabolites, platinum-based cytotoxic drugs, hormonal antagonists, antiestrogens, tamoxifen, doxorubicin, L-asparaginase, dacarbazine, amsacrine, procarbazine, hexamethylmelamine and mitoxantrone.

39. The method of claim 38 wherein the anti-bacterial agent is selected from the group consisting of a heavy metal and an antibiotic.

40. The method of claim 36 wherein the pharmaceutical agent is an inflammatory agent.

41. The method of claim 36 wherein the pharmaceutical agent is an analgesic.

42. The method of claim 36 wherein the pharmaceutical agent is a chemotherapeutic substance.

43. The method of claim 1 wherein the biocompatible additive is a hormone.

44. The method of claim 43 wherein the hormone is selected from the group consisting of insulin, atrial natriuretic factor, calcitonin, vasopressin, and relaxin.

45. The method of claim 43 wherein the hormone is selected from the group consisting of an estrogenic hormone, a progestational hormone, and an androgenic hormone.

46. The method of claim 1 wherein the biocompatible additive is an antibiotic.

47. The method of claim 46 wherein the antibiotic is an aminoglycoside.

48. The method of claim 46 wherein the antibiotic is an amphenicol.

49. The method of claim 48 wherein the amphenicol is chloramphenicol.

50. The method of claim 46 wherein the antibiotic is a β-Lactams antibiotic.

51. The method of claim 46 wherein the antibiotic is a penicillin.

52. The method of claim 51 wherein the penicillin is ampicillin.

53. The method of claim 46 wherein the antibiotic is a peptide antibiotic.

54. The method of claim 46 wherein the antibiotic is a tetracycline antibiotic.

55. The method of claim 54 wherein the tetracycline antibiotic is tetracycline.

56. The method of claim 1 further comprising the step of combining the biocompatible hydroxyapatite formulation with an antibiotic.

57. The method of claim 1 further comprising the step of combining the biocompatible hydroxyapatite formulation with heparanase.

58. The method of claim 1 wherein said mixture has a solid-to-liquid ratio of from about 1:1 to about 5:1.

59. The method of claim 1 wherein the liquid phase comprises a liquid select from the group consisting of water, saline, a weakly acidic solution, a biocompatible buffer solution, serum, and plasma.

60. The method of claim 1 wherein the liquid phase is supplemented with one or more components selected from the group consisting of proteoglycan, hyaluronic acid, protein, serum albumin, carbohydrates, granular sugar, a synthetic material, polyethylene glycol, ionic agents, non-cross-linked collagen, and glycerin.

61. The method of claim 1 wherein the precipitation step occurs at a temperature in the range of from about 4°C to about 50°C.

62. The method of claim 1 wherein the precipitation step occurs at a temperature in the range of from about 15°C to about 42°C.

63. The method of claim 1 further comprising the step of hardening the biocompatible hydroxyapatite formulation to a substantially uniform crystallinity.

64. The method of claim 1 further comprising the step of hardening the biocompatible hydroxyapatite formulation to a substantially uniform porosity.

65. The method of claim 1 further comprising the steps of shaping the biocompatible hydroxyapatite formulation into a structure.

66. The method of claim 65 wherein the structure is a wound dressing, a bone substitute, a cartilagineous substitute, or a soft tissue substitute.

67. The method of claim 65 wherein the structure is a sheet, a membrane, a coating, or a biological prosthesis.

68. The method of claim 67 wherein the membrane has a thickness in the range of from about 1 mm to about 7 mm.

69. The method of claim 65 wherein the structure is a granular block.

70. A biocompatible hydroxyapatite formulation prepared by the method of claim 1.

71. The formulation of claim 1 wherein the biocompatible additive is lyophilized to a powder.

72. The formulation of claim 71 wherein the powder is stable for greater than about 3 months.

73. The formulation of claim 1 further comprising a pharmaceutically acceptable carrier.

74. The formulation of claim 73 wherein the pharmaceutically acceptable carrier is selected from the group consisting of water, glycerol, glycols, saccharide, polysaccharides, oils, salts and fatty acids.

75. A method for treating a patient comprising the steps of:

a) preparing a base combination of calcium phosphate salts;

b) preparing a liquid phase;

c) providing a biocompatible additive;

d) combining the base combination of calcium phosphate salts, the liquid phase and the biocompatible additive to form a mixture;

e) precipitating the biocompatible hydroxyapatite formulation from the mixture; and

f) administering the precipitated biocompatible hydroxyapatite formulation to the patient.

76. The method of claim 75 wherein the combining step comprises:

a) adding the biocompatible additive to the liquid phase to form an augmented liquid phase; and

b) mixing the augmented liquid phase with the base combination of calcium phosphate salts.

77. The method of claim 75 wherein the combining step comprises:

a) adding the biocompatible additive to the base combination of calcium phosphate salts to form an augmented combination of calcium phosphate salts; and

b) mixing the liquid phase with the augmented combination of calcium phosphate salts.

78. The method of claim 75 wherein the combining step comprises simultaneously combining the base combination of calcium phosphate salts, the biocompatible additive and the liquid phase.

79. The method of claim 75 wherein the precipitated biocompatible hydroxyapatite formulation is absorbed by the patient after administration.

80. The method of claim 75 wherein the precipitated biocompatible hydroxyapatite formulation is non-immunogenic to the patient.

81. The method of claim 75 wherein the biocompatible additive is released from the hydroxyapatite formulation in a timed-release fashion.

82. The method of claim 81 wherein less than about 20% of said additive is released in about 24 hours.

83. The method of claim 81 wherein more than about 90% of said additive is released in about 30 days.

84. The method of claim 75 further comprising the step of forming the precipitated biocompatible hydroxyapatite formulation into a paste.

85. The method of claim 84 wherein the paste is glue, a dressing, a biological patch, a delivery vehicle, an absorbent, a coating or a shield.

86. The method of claim 85 wherein the biological delivery vehicle is a contraceptive device.

87. The method of claim 85 wherein the glue is a bone glue.

88. The method of claim 75 further comprising the steps of forming the precipitated hydroxyapatite formulations into a shape and administering the shape to the patient.

89. The method of claim 88 wherein the shape is an medical prosthesis.

90. The method of claim 89 wherein the medical prosthesis is administered cutaneously, subcutaneously, or intramuscularly.

91. The method of claim 75 wherein the precipitated formulation is administered by covering, implanting, or injecting.

92. A kit for precipitating a biocompatible hydroxyapatite formulation comprising:

a predetermined amount of a base combination of calcium phosphate salts;

a predetermined amount of a liquid phase; and

a predetermined amount of a biocompatible additive,

wherein the base combination of calcium phosphate salts, the biocompatible additive and the liquid phase may be combined to form a mixture which precipitates the biocompatible hydroxyapatite formulation.

93. The kit of claim 92, wherein the base combination of calcium phosphate salts, the biocompatible additive and the liquid phase may be simultaneously combined to precipitate the biocompatible hydroxyapatite formulation.

94. The kit of claim 92, wherein the biocompatible additive may be added to the liquid phase to form an augmented liquid phase, and wherein the augmented liquid phase may be added to the base combination of calcium phosphate salts to precipitate the biocompatible hydroxyapatite formulation.

95. The kit of claim 92, wherein the biocompatible additive may be added to the base combination of calcium phosphate salts to form an augmented combination of calcium phosphate salts, and wherein the augmented combination

of calcium phosphate salts may be added to the liquid phase to precipitate the biocompatible hydroxyapatite formulation.

96. The kit of claim 92 wherein the biocompatible additive is in a lyophilized state.

97. The kit of claim 92 wherein the base combination of calcium phosphate salts is in a first container, wherein the liquid phase is in a second container, and wherein the biocompatible additive is in a third container.

98. The kit of claim 92 wherein two or more of the basc combination of calcium phosphate salts, the liquid phase and the biocompatible additive are in the same container.

99. A kit for precipitating a biocompatible hydroxyapatite formulation comprising:

a predetermined amount of a base combination of calcium phosphate salts; and

a predetermined amount of an augmented liquid phase,

wherein the augmented liquid phase comprises a liquid phase and a biocompatible additive, and wherein the augmented liquid phase may be added to the base combination of calcium phosphate salts to precipitate the biocompatible hydroxyapatite formulation.

100. The kit of claim 99 wherein the base combination of calcium phosphate salts is in a first container, and wherein the augmented liquid phase is in a second container.

101. A kit for precipitating a biocompatible hydroxyapatite formulation comprising:

a predetermined amount of an augmented combination of calcium phosphate salts; and

a predetermined amount of a liquid phase,

wherein the augmented combination of calcium phosphate salts comprises a base combination of calcium phosphate salts and a biocompatible additive, and wherein the liquid phase may be added to the augmented combination of calcium phosphate salts to precipitate the biocompatible hydroxyapatite formulation.

102. A method for preparing a biocompatible hydroxyapatite formulation comprising the steps of:

a) preparing a base combination of calcium phosphate salts;

b) preparing a liquid phase;

c) providing an adhesive agent;

d) combining the base combination of calcium phosphate salts, the liquid phase and the adhesive agent to form a mixture; and

e) precipitating the biocompatible hydroxyapatite formulation from the mixture.

103. The method of claim 102 wherein the combining step comprises:

a) adding the adhesive agent to the liquid phase to form an augmented liquid phase; and

b) mixing the augmented liquid phase with the base combination of calcium phosphate salts.

104. The method of claim 102 wherein the combining step comprises:

a) adding the adhesive agent to the base combination of calcium phosphate salts to form an augmented combination of calcium phosphate salts; and

b) mixing the liquid phase with the augmented combination of calcium phosphate salts.

105. The method of claim 102 wherein the combining step comprises simultaneously combining the base combination of calcium phosphate salts, the adhesive agent and the liquid phase.

106. The method of claim 102, wherein said precipitating step occurs in vivo.

107. The method of claim 102, wherein said precipitating step occurs ex vivo.

108. The method of claim 102, wherein said precipitating step occurs partially ex vivo and partially in vivo.

109. The method of claim 102, further comprising the step of permitting the precipitated biocompatible hydroxyapatite formulation to be resorbed or degraded in vivo.

110. The method of claim 102 wherein the base combination comprises two salts and wherein one of the two salts is tetracalcium phosphate and the other of the two salts is selected from the group consisting of $CaHPO_4 \cdot 2H_2O$, $CaHPO_4$, $Ca_8H_2(PO_4)_6 \cdot 5H_2O$, β-$Ca_3(PO_4)_2$, α-$Ca_3(PO_4)_2$, and modified $Ca_3(PO_4)_2$.

111. The method of claim 110 wherein the modified $Ca_3(PO_4)_2$ is tricalcium phosphate modified by protons or up to about 10 wt % magnesium.

112. The method of claim 102 wherein the adhesive agent is selected from the group consisting of integrins, extracellular matrix proteins, leukocyte adhesion proteins, collagen, albumins, bone proteins, osteonectins, cell surface receptor proteins, bone *gla* protein, and matrix *gla* protein.

113. The method of claim 102 further comprising the step of combining the biocompatible hydroxyapatite formulation with an antibiotic.

114. The method of claim 102 further comprising the step of combining the biocompatible hydroxyapatite formulation with heparanase.

115. The method of claim 102 further comprising the step of combining the biocompatible hydroxyapatite formulation with a growth factor.

116. The method of claim 115 wherein the growth factor is selected from the group consisting of an osteogenic factor, a bone morphogenic factor, a protein and a chondrogenic factor.

117. The method of claim 115 wherein the biocompatible hydroxyapatite formulation contains from about 10μg to about 100μg of the growth factor per cubic centimeter of the biocompatible hydroxyapatite formulation.

118. The method of claim 115 wherein the biocompatible hydroxyapatite formulation contains from about 100ug to about 500μg of the growth factor per cubic centimeter of the biocompatible hydroxyapatite formulation.

119. The method of claim 102 wherein said mixture has a solid-to-liquid ratio of from about 1:1 to about 5:1.

120. The method of claim 102 wherein the liquid phase comprises a liquid select from the group consisting of water, saline, a weakly acidic solution, a biocompatible buffer solution, serum, and plasma.

121. The method of claim 102 wherein the liquid phase is supplemented with one or more components selected from the group consisting of proteoglycan, hyaluronic acid, protein, serum albumin, carbohydrates, granular sugar, a synthetic material, polyethylene glycol, ionic agents, non-cross-linked collagen, and glycerin.

122. The method of claim 102 wherein the precipitation step occurs at a temperature in the range of from about 4°C to about 50°C.

123. The method of claim 102 wherein the precipitation step occurs at a temperature in the range of from about 15°C to about 42°C.

124. The method of claim 102 further comprising the step of hardening the biocompatible hydroxyapatite formulation to a substantially uniform crystallinity.

125. The method of claim 102 further comprising the step of hardening the biocompatible hydroxyapatite formulation to a substantially uniform porosity.

126. The method of claim 102 further comprising the steps of shaping the biocompatible hydroxyapatite formulation into a structure.

127. The method of claim 126 wherein the structure is a wound dressing, a bone substitute, a cartilagineous substitute, or a soft tissue substitute.

128. The method of claim 126 wherein the structure is a sheet, a membrane, a coating, or a biological prosthesis.

129. The method of claim 128 wherein the membrane has a thickness in the range of from about 1 mm to about 7 mm.

130. The method of claim 126 wherein the structure is a granular block.

131. The method of claim 102 wherein the precipitating step comprises precipitating a first component of the biocompatible hydroxyapatite formulation and precipitating a second component of the biocompatible hydroxyapatite formulation, wherein said first component has a slower resorption rate than said second component.

132. A biocompatible hydroxyapatite formulation prepared by the method of claim 102.

133. The formulation of claim 102 wherein the adhesive agent is lyophilized to a powder.

134. The formulation of claim 133 wherein the powder is stable for greater than about 3 months.

135. The method of claim 102 further comprising the step of hardening the biocompatible hydroxyapatite formulation.

136. The method of claim 135 wherein the hardened biocompatible hydroxyapatite formulation has a tensile strength of at least 20 MPa.

137. The method of claim 135 wherein the hardened biocompatible hydroxyapatite formulation has a tensile strength of at least 60 MPa.

138. The method of claim 135 wherein the hardened biocompatible hydroxyapatite formulation has a compressive strength of at least 10 MPa.

139. The method of claim 135 wherein the hardened biocompatible hydroxyapatite formulation has a compressive strength of at least 50 MPa.

140. A layered device comprising a first layer and a second layer, wherein the first layer comprises a synthetic polymer, and wherein the second layer comprises the biocompatible hydroxyapatite formulation of claim 102.

141. A method for treating a patient comprising the steps of:

a) preparing a base combination of calcium phosphate salts;

b) preparing a liquid phase;

c) providing an adhesive agent;

d) combining the base combination of calcium phosphate salts, the liquid phase and the biocompatible additive to form a mixture;

e) precipitating the biocompatible hydroxyapatite formulation from the mixture; and

f) administering the precipitated biocompatible hydroxyapatite formulation to the patient.

142. The method of claim 141 wherein the combining step comprises:

a) adding the adhesive agent to the liquid phase to form an augmented liquid phase; and

b) mixing the augmented liquid phase with the base combination of calcium phosphate salts.

143. The method of claim 141 wherein the combining step comprises:

a) adding the adhesive agent to the base combination of calcium phosphate salts to form an augmented combination of calcium phosphate salts; and

b) mixing the liquid phase with the augmented combination of calcium phosphate salts.

144. The method of claim 141 wherein the combining step comprises simultaneously combining the base combination of calcium phosphate salts, the adhesive agent and the liquid phase.

145. The method of claim 141 wherein the precipitated biocompatible hydroxyapatite formulation is absorbed by the patient after administration.

146. The method of claim 141 wherein the precipitated biocompatible hydroxyapatite formulation is non-immunogenic to the patient.

147. The method of claim 141 wherein the adhesive agent is released from the hydroxyapatite formulation in a timed-release fashion.

148. The method of claim 147 wherein less than about 20% of said adhesive agent is released in about 24 hours.

149. The method of claim 147 wherein more than about 90% of adhesive agent is released in about 30 days.

150. The method of claim 141 further comprising the step of forming the precipitated biocompatible hydroxyapatite formulation into a paste.

151. The method of claim 150 wherein the paste is glue, a dressing, a biological patch, a delivery vehicle, an absorbent, a coating or a shield.

152. The method of claim 151 wherein the biological delivery vehicle is a contraceptive device.

153. The method of claim 151 wherein the glue is a bone glue.

154. The method of claim 141 further comprising the steps of forming the precipitated hydroxyapatite formulations into a shape and administering the shape to the patient.

155. The method of claim 154 wherein the shape is an medical prosthesis.

156. The method of claim 155 wherein the medical prosthesis is administered cutaneously, subcutaneously, or intramuscularly.

157. The method of claim 141 wherein the precipitated formulation is administered by covering, implanting, or injecting.

158. A kit for precipitating a biocompatible hydroxyapatite formulation comprising:

a predetermined amount of a base combination of calcium phosphate salts;

a predetermined amount of a liquid phase; and

a predetermined amount of an adhesive agent,

wherein the base combination of calcium phosphate salts, the adhesive agent and the liquid phase may be combined to form a mixture which precipitates the biocompatible hydroxyapatite formulation.

159. The kit of claim 158, wherein the base combination of calcium phosphate salts, the adhesive agent and the liquid phase may be simultaneously combined to precipitate the biocompatible hydroxyapatite formulation.

160. The kit of claim 158, wherein the adhesive agent may be added to the liquid phase to form an augmented liquid phase, and wherein the augmented liquid phase may be added to the base combination of calcium phosphate salts to precipitate the biocompatible hydroxyapatite formulation.

161. The kit of claim 158, wherein the adhesive agent may be added to the base combination of calcium phosphate salts to form an augmented combination of calcium phosphate salts, and wherein the augmented combination of calcium phosphate salts may be added to the liquid phase to precipitate the biocompatible hydroxyapatite formulation.

162. The kit of claim 158 wherein the adhesive agent is in a lyophilized state.

163. The kit of claim 158 wherein the base combination of calcium phosphate salts is in a first container, wherein the liquid phase is in a second container, and wherein the adhesive agent is in a third container.

164. The kit of claim 158 wherein two or more of the base combination of calcium phosphate salts, the adhesive agent and the liquid phase are in the same container.

165. A kit for precipitating a biocompatible hydroxyapatite formulation comprising:

a predetermined amount of a base combination of calcium phosphate salts; and

a predetermined amount of an augmented liquid phase,

wherein the augmented liquid phase comprises a liquid phase and an adhesive agent, and wherein the augmented liquid phase may be added to the base combination of calcium phosphate salts to precipitate the biocompatible hydroxyapatite formulation.

166. The kit of claim 165 wherein the base combination of calcium phosphate salts is in a first container, and wherein the augmented liquid phase is in a second container.

167. A kit for precipitating a biocompatible hydroxyapatite formulation comprising:

a predetermined amount of an augmented combination of calcium phosphate salts; and

a predetermined amount of a liquid phase,

wherein the augmented combination of calcium phosphate salts comprises a base combination of calcium phosphate salts and an adhesive agent, and wherein the liquid phase may be added to the augmented combination of calcium phosphate salts to precipitate the biocompatible hydroxyapatite formulation.

168. A method for preparing a biocompatible hydroxyapatite formulation comprising the steps of:

a) preparing a base combination of calcium phosphate salts;

b) preparing a liquid phase;

c) providing an electrical stimulus enhancer;

d) combining the base combination of calcium phosphate salts, the liquid phase, and the electrical stimulus enhancer to form a mixture; and

e) precipitating the biocompatible hydroxyapatite formulation from the mixture.

169. The method of claim 168 wherein the combining step comprises:

a) adding the electrical stimulus enhancer to the liquid phase to form an augmented liquid phase; and

b) mixing the augmented liquid phase with the base combination of calcium phosphate salts.

170. The method of claim 168 wherein the combining step comprises:

a) adding the electrical stimulus enhancer to the base combination of calcium phosphate salts to form an augmented combination of calcium phosphate salts; and

b) mixing the liquid phase with the augmented combination of calcium phosphate salts.

171. The method of claim 168 wherein the combining step comprises simultaneously combining the base combination of calcium phosphate salts, the electrical stimulus enhancer and the liquid phase.

172. The method of claim 168, wherein said precipitating step occurs in vivo.

173. The method of claim 168, wherein said precipitating step occurs ex vivo.

174. The method of claim 168, wherein said precipitating step occurs partially ex vivo and partially in vivo.

175. The method of claim 168, further comprising the step of permitting the precipitated biocompatible hydroxyapatite formulation to be resorbed or degraded in vivo.

176. The method of claim 168 wherein the base combination comprises two salts and wherein one of the two salts is tetracalcium phosphate and the other of

the two salts is selected from the group consisting of $CaHPO_4 \cdot 2H_2O$, $CaHPO_4$, $Ca_8H_2(PO_4)_6 \cdot 5H_2O$, β-$Ca_3(PO_4)_2$, α-$Ca_3(PO_4)_2$, and modified $Ca_3(PO_4)_2$.

177. The method of claim 176 wherein the modified $Ca_3(PO_4)_2$ is tricalcium phosphate modified by protons or up to about 10 wt % magnesium.

178. The method of claim 168 wherein the electrical stimulus enhancer is a paramagnetic material.

179. The method of claim 178 wherein the paramagnetic material is selected from the group consisting of iron, iron ammonium alum, uranium, platinum, and aluminum.

180. The method of claim 168 wherein the electrical stimulus enhancer is a diamagnetic material.

181. The method of claim 180 wherein the diamagnetic material is selected from the group consisting of bismuth, mercury, silver, carbon, diamond, lead, and copper.

182. The method of claim 168 wherein the electrical stimulus enhancer is a conductive material.

183. The method of claim 182 wherein the conductive material is selected from the group consisting of silver, copper, aluminum, and tungsten.

184. The method of claim 168 wherein the electrical stimulus enhancer is an insulator.

185. The method of claim 184 wherein the insulator is selected from the group consisting of glass, lucite, mica, quartz, and polytetrafluoroethylene.

186. The method of claim 168 further comprising the step of combining the biocompatible hydroxyapatite formulation with an antibiotic.

187. The method of claim 168 further comprising the step of combining the biocompatible hydroxyapatite formulation with heparanase.

188. The method of claim 168 wherein the mixture has a solid-to-liquid ratio of from about 1:1 to about 5:1.

189. The method of claim 168 wherein the liquid phase comprises a liquid select from the group consisting of water, saline, a weakly acidic solution, a biocompatible buffer solution, serum, and plasma.

190. The method of claim 168 wherein the liquid phase is supplemented with one or more components selected from the group consisting of proteoglycan, hyaluronic acid, protein, serum albumin, carbohydrates, granular sugar, a synthetic material, polyethylene glycol, ionic agents, non-cross-linked collagen, and glycerin.

191. The method of claim 168 wherein the precipitation step occurs at a temperature in the range of from about 4°C to about 50°C.

192. The method of claim 168 wherein the precipitation step occurs at a temperature in the range of from about 15°C to about 42°C.

193. The method of claim 168 further comprising the step of hardening the biocompatible hydroxyapatite formulation to a substantially uniform crystallinity.

194. The method of claim 168 further comprising the step of hardening the biocompatible hydroxyapatite formulation to a substantially uniform porosity.

195. The method of claim 168 further comprising the steps of shaping the biocompatible hydroxyapatite formulation into a structure.

196. The method of claim 195 wherein the structure is a wound dressing, a bone substitute, a cartilagineous substitute, or a soft tissue substitute.

197. The method of claim 195 wherein the structure is a sheet, a membrane, a coating, or a biological prosthesis.

198. The method of claim 197 wherein the membrane has a thickness in the range of from about 1 mm to about 7 mm.

199. The method of claim 195 wherein the structure is a granular block.

200. The method of claim 168 wherein the precipitating step comprises precipitating a first component of the biocompatible hydroxyapatite formulation and precipitating a second component of the biocompatible hydroxyapatite

formulation, wherein said first component has a slower resorption rate than said second component.

201. A biocompatible hydroxyapatite formulation prepared by the method of claim 168.

202. The formulation of claim 168 wherein the electrical stimulus enhancer is lyophilized to a powder.

203. The formulation of claim 202 wherein the powder is stable for greater than about 3 months.

204. The formulation of claim 168 further comprising a pharmaceutically acceptable carrier.

205. The formulation of claim 204 wherein the pharmaceutically acceptable carrier is selected from the group consisting of water, glycerol, glycols, saccharide, polysaccharides, oils, salts and fatty acids.

206. The method of claim 168 further comprising the steps of:

a) providing a biocompatible additive; and

b) combining the biocompatible additive with the base combination of calcium phosphate salts, the electrical stimulus enhancer and the liquid phase.

207. A method for treating a patient comprising the steps of:

a) preparing a base combination of calcium phosphate salts;

b) preparing a liquid phase;

c) providing an electrical stimulus enhancer;

d) combining the base combination of calcium phosphate salts, the liquid phase and the electrical stimulus enhancer to form a mixture;

e) precipitating the biocompatible hydroxyapatite formulation from the mixture; and

f) administering the precipitated biocompatible hydroxyapatite formulation to the patient.

208. The method of claim 207 wherein the combining step comprises:

a) adding the electrical stimulus enhancer to the liquid phase to form an augmented liquid phase; and

b) mixing the augmented liquid phase with the base combination of calcium phosphate salts.

209. The method of claim 207 wherein the combining step comprises:

a) adding the electrical stimulus enhancer to the base combination of calcium phosphate salts to form an augmented combination of calcium phosphate salts; and

b) mixing the liquid phase with the augmented combination of calcium phosphate salts.

210. The method of claim 207 wherein the combining step comprises simultaneously combining the base combination of calcium phosphate salts, the electrical stimulus enhancer and the liquid phase.

211. The method of claim 207 wherein the precipitated biocompatible hydroxyapatite formulation is absorbed by the patient after administration.

212. The method of claim 207 wherein the precipitated biocompatible hydroxyapatite formulation is non-immunogenic to the patient.

213. The method of claim 207 further comprising the steps of:

a) providing a biocompatible additive; and

b) combining the biocompatible additive with the base combination of calcium phosphate salts, the electrical stimulus enhancer and the liquid phase.

214. The method of claim 213 wherein the biocompatible additive is released from the precipitated biocompatible hydroxyapatite formulation in a timed-release fashion.

215. The method of claim 214 wherein less than about 20% of said additive is released in about 24 hours.

216. The method of claim 214 wherein more than about 90% of said additive is released in about 30 days.

217. The method of claim 207 further comprising the step of forming the precipitated biocompatible hydroxyapatite formulation into a paste.

218. The method of claim 217 wherein the paste is glue, a dressing, a biological patch, a delivery vehicle, an absorbent, a coating or a shield.

219. The method of claim 218 wherein the biological delivery vehicle is a contraceptive device.

220. The method of claim 218 wherein the glue is a bone glue.

221. The method of claim 207 further comprising the steps of forming the precipitated hydroxyapatite formulations into a shape and administering the shape to the patient.

222. The method of claim 221 further comprising the step of electrically or electromagnetically stimulating the shape.

223. The method of claim 221 wherein the shape is an medical prosthesis.

224. The method of claim 223 wherein the medical prosthesis is administered cutaneously, subcutaneously, or intramuscularly.

225. The method of claim 207 wherein the precipitated formulation is administered by covering, implanting, or injecting.

226. The method of claim 207 further comprising the step of electrically or electromagnetically stimulating the biocompatible hydroxyapatite formulation.

227. A kit for precipitating a biocompatible hydroxyapatite formulation comprising:

a predetermined amount of a base combination of calcium phosphate salts;

a predetermined amount of an electrical stimulus enhancer; and

a predetermined amount of a liquid phase,

wherein the base combination of calcium phosphate salts, the electrical stimulus enhancer and the liquid phase may be combined to form a mixture which precipitates the biocompatible hydroxyapatite formulation.

228. The kit of claim 227, wherein the base combination of calcium phosphate salts, the electrical stimulus enhancer and the liquid phase may be simultaneously combined to precipitate the biocompatible hydroxyapatite formulation.

229. The kit of claim 227, wherein the electrical stimulus enhancer may be added to the liquid phase to form an augmented liquid phase, and wherein the augmented liquid phase may be added to the base combination of calcium phosphate salts to precipitate the biocompatible hydroxyapatite formulation.

230. The kit of claim 227, wherein the electrical stimulus enhancer may be added to the base combination of calcium phosphate salts to form an augmented combination of calcium phosphate salts, and wherein the augmented combination of calcium phosphate salts may be added to the liquid phase to precipitate the biocompatible hydroxyapatite formulation.

231. The kit of claim 227 wherein the base combination of calcium phosphate salts is in a first container, wherein the liquid phase is in a second container, and wherein the electrical stimulus enhancer is in a third container.

232. A kit for precipitating a biocompatible hydroxyapatite formulation comprising:

a predetermined amount of a base combination of calcium phosphate salts; and

a predetermined amount of an augmented liquid phase,

wherein the augmented liquid phase comprises a liquid phase and an electrical stimulus enhancer, and wherein the augmented liquid phase may be added to the base combination of calcium phosphate salts to precipitate the biocompatible hydroxyapatite formulation.

233. The kit of claim 232 wherein the base combination of calcium phosphate salts is in a first container, and wherein the augmented liquid phase is in a second container.

234. A kit for precipitating a biocompatible hydroxyapatite formulation comprising:

a predetermined amount of an augmented combination of calcium phosphate salts; and

a predetermined amount of a liquid phase,

wherein the augmented combination of calcium phosphate salts comprises a base combination of calcium phosphate salts and an electrical stimulus enhancer, and wherein the liquid phase may be added to the augmented combination of calcium phosphate salts to precipitate the biocompatible hydroxyapatite formulation.

235. The kit of claim 227 further comprising a biocompatible additive which may be combined with the base combination of calcium phosphate salts, the electrical stimulus enhancer and the liquid phase.

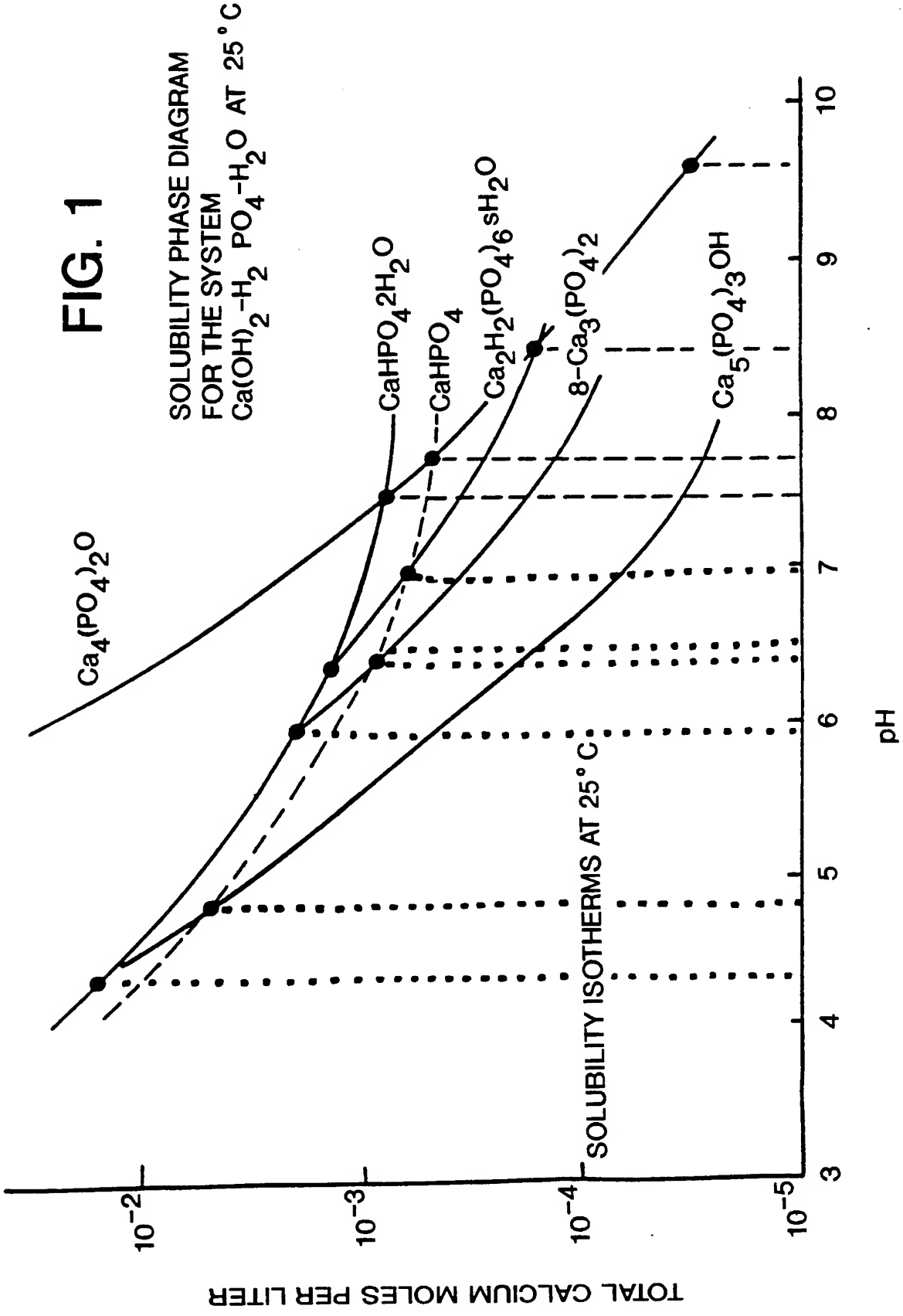
236. The kit of claim 227 wherein two or more of the base combination of calcium phosphate salts, the electrical stimulus enhancer and the liquid phase are in the same container.

237. The kit of claim 235 wherein the biocompatible additive is in a fourth container.

238. The kit of claim 235 wherein the biocompatible additive is in a lyophilized state.

239. The kit of claim 235 wherein two or more of the base combination of calcium phosphate salts, the liquid phase, the electrical stimulus enhancer and the biocompatible additive are in the same container.

FIG. 1


SOLUBILITY PHASE DIAGRAM
FOR THE SYSTEM
$Ca(OH)_2$-H_2 PO_4-H_2O AT 25 °C
$Ca_4(PO_4)_2O$
$CaHPO_4 2H_2O$
$CaHPO_4$
$Ca_2H_2(PO_4)_6 sH_2O$
8-$Ca_3(PO_4)_2$
$Ca_5(PO_4)_3OH$
SOLUBILITY ISOTHERMS AT 25 °C
TOTAL CALCIUM MOLES PER LITER
10^{-2}
10^{-3}
10^{-4}
10^{-5}
pH
3
4
5
6
7
8
9
10

INTERNATIONAL SEARCH REPORT

International Application No
PC.,US 96/08652

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61L27/00 A61L25/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO,A,94 20064 (AMERICAN DENTAL ASS) 15 September 1994 see page 23, line 14 - line 21; claims	1-9
X	DATABASE WPI Week 9515 Derwent Publications Ltd., London, GB; AN 95-109588 XP002016014 & JP,A,07 031 673 (ASAHI OPTICAL CO LTD) , 3 February 1995 see abstract	1-9
X	EP,A,0 389 629 (NITTA GELATIN KK) 3 October 1990 see page 55; claims; examples	1

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 October 1996	04. 11. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	ESPINOSA, M

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US,A,5 149 368 (LIU SUNG-TSUEN ET AL) 22 September 1992 cited in the application see claims; examples 1-5	1-239
Y	US,E,RE33221 (W.E. BROWN ET AL.) 22 May 1990 cited in the application see the whole document	1-239
A	EP,A,0 538 913 (NITTA GELATIN KK) 28 April 1993 see claims	1
A	EP,A,0 323 632 (ASAHI OPTICAL CO LTD) 12 July 1989 see claims	1
A	GB,A,2 248 232 (MITSUBISHI MATERIALS CORP) 1 April 1992 see claims; examples	1

INTERNATIONAL SEARCH REPORT

I national application No.
PCT/US 96/08652

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 75-91 ; 141-157 ; 207-226
because they relate to subject matter not required to be searched by this Authority, namely:
Remark : although claims 75-91, 141-157, 207-226 are directed to a method of treatment of the human or animal body the search has been carried out and based on the alleged effects of the compounds/compositions.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PC../US 96/08652

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		DE-T-	68915265	05-01-95
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		DE-A-	4132331	09-04-92
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